Pat1 proteins: a life in translation, translation repression and mRNA decay

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Abstract
Pat1 proteins are conserved across eukaryotes. Vertebrates have evolved two Pat1 proteins paralogues, whereas invertebrates and yeast only possess one such protein. Despite their lack of known domains or motifs, Pat1 proteins are involved in several key post-transcriptional mechanisms of gene expression control. In yeast, Pat1p interacts with translating mRNPs (messenger ribonucleoproteins), and is responsible for translational repression and decapping activation, ultimately leading to mRNP degradation. Drosophila HPat and human Pat1b (PatL1) proteins also have conserved roles in the 5′→3′ mRNA decay pathway. Consistent with their functions in silencing gene expression, Pat1 proteins localize to P-bodies (processing bodies) in yeast, Drosophila, Caenorhabditis elegans and human cells. Altogether, Pat1 proteins may act as scaffold proteins allowing the sequential binding of repression and decay factors on mRNPs, eventually leading to their degradation. In the present mini-review, we present the current knowledge on Pat1 proteins in the context of their multiple functions in post-transcriptional control.

Yeast Pat1p and 5′→3′ mRNA decay
The transition between actively translating mRNAs and those targeted for degradation is a critical step in post-transcriptional gene regulation in eukaryotes (reviewed in [1]). Indeed, when translation initiation is decreased by different means, the rates of deadenylation and decapping of mRNAs increase [2]. An mRNP (messenger ribonucleoprotein) is thought to first exit translation and assemble into a repressed mRNP which will be targeted for decay [3]. In eukaryotes, mRNAs are degraded via the 5′→3′ mRNA decay or the 3′→5′ exosome pathways. The 5′→3′ mRNA decay pathway is initiated by the deadenylation of the mRNA by the Ccr4/Pop2/Not1–5p complex; then followed by the removal of the m7G cap structure by the decapping complex, Dcp1/2; and ultimately leading to the 5′→3′ exonucleotic digestion of the mRNA by Xrn1 (reviewed in [1]). This pathway can take place in P-bodies (processing bodies), where non-translating mRNP, decay factors, RNA-binding proteins and miRNP (microribonucleoprotein) localize (reviewed in [4,5]). Although P-bodies and their components are conserved across eukaryotes, suggesting a shared mechanism by which mRNAs are repressed and committed for degradation, current evidence indicates that visible foci are not required for mRNA decay [6].

Pat1p was first identified as a protein associated with topoisomerase II [7]. Since then, no other studies have focused on this nuclear function. Rather, Pat1p has been reported to be involved in multiple post-transcriptional processes in yeast, where it has predominantly been studied, until very recently. Pat1p mutant or deletion strains were shown to increase the half-lives of mRNA reporters [8–10], resulting in deadenylated, but capped, forms, suggesting a role for Pat1p at the level of decapping [8,9]. Like several other decapping/decay factors including Dcp1, LSm1 (part of the LSm1–7 complex responsible for decapping activation) and Xrn1, Pat1p mutant/deletion strains were able to suppress the lethality of the PABP1 [poly(A)-binding protein 1] deletion, by an unknown mechanism [8,11,12].

Is Pat1p implicated in the mRNP rearrangement from a translating mRNAs to one targeted for degradation?

The detailed mechanisms by which yeast Pat1p activates decapping remain unclear. An overall summary of the different post-transcriptional steps at which Pat1p functions is discussed below and illustrated in Figure 1.

There is evidence to show that Pat1p plays a role in translation (Figure 1). Pat1p associates with the initiation factors eIF (eukaryotic initiation factor) 4E, eIF4G and PABP1, in an RNA-dependent manner [3]. Moreover, a fraction of Pat1p is associated with polysomes [10], in particular with the 40–48S ribosomal subunits [11], thus suggesting a role in translation initiation. Overall, then, at least some Pat1p associates with translating mRNP.

A critical step in mRNA decay is the repression of translation [3]. Overexpression of Pat1p leads to a global repression of translation and an increase in P-body abundance [13]. Its deletion together with deletion of Dhh1 (DDX6/p54 RNA helicase), also a decapping activator, blocks mRNAs on polysomes upon glucose deprivation. Therefore Pat1p and Dhh1 together act as general repressors of translation, although, independently, they only have a modest effect on protein synthesis [13]. Consistent with this observation, Pat1p is not required for P-body formation, since P-bodies

Key words: deadenylation, LSm1–7 complex, Pat1, Pat1b, p54/DDX6/Dhh1/Me31B, vnon.

Abbreviations used: Dcp, decapping enzyme; eIF, eukaryotic initiation factor; mRNP, messenger ribonucleoprotein; mRNP, messenger ribonucleoprotein; mRNP, messenger ribonucleoprotein; PABP, poly(A)-binding protein; P-body, processing body.

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Pat1p is thought to associate with actively translating mRNPs and even perhaps to act at the translation initiation step. Upon a signal targeting the mRNA for degradation, Pat1p represses the mRNA from translation (either directly or via other protein partners) and the deadenylation of the mRNA is triggered. Dhh1 and Pat1p may play a redundant role in translational repression. During or after deadenylation, the repressed mRNP is likely to accumulate in P-bodies where it undergoes degradation. The signals leading to the commitment of a repressed mRNP into a mRNP targeted for decay are not well understood. However, mRNA deadenylation is a critical step in committing the mRNA for decay. As discussed above, Pat1p probably acts after deadenylation, at the level of decapping [8,9]. Indeed, Pat1p interacts with Edc3, an enhancer of decapping, with Dcp1, the decapping enzyme, and tightly with the LSm1–7 complex [9,10,16–18]. Pat1p is thought to recruit the LSm1–7 complex to P-bodies in order to trigger decapping [15] (Figure 1). The purified LSm1–7–Pat1 complex binds preferentially at the 3′-end of oligoadenylated mRNAs rather than polyadenylated mRNAs [3,19–21]. Furthermore, the LSm1–7–Pat1 complex interacts with the decapping complex, Dcp1/2, in an RNA-dependent manner, probably via deadenylated mRNAs [3]. The activation of decapping by the LSm1–7–Pat1 complex was shown to occur at a different step than that involving the competition between Dcp1 and eIF4E [22]. Overall, Pat1p and the LSm1–7 complex may promote decapping by stabilizing the Dcp1/2 complex on the mRNA [3] (Figure 1). Once the cap is removed, transcripts are digested by Xrn1. The LSm1–7–Pat1 complex appears to remain bound on the mRNA while the 5′→3′ exonucleic digestion takes place [3] (Figure 1), consistent with the LSm1–7 complex interacting with Xrn1 [9,17,18].

An additional role, independent of their role in decapping, was attributed to the LSm1–7–Pat1 complex in preventing 3′-end trimming of mRNAs, corresponding to the shortening of the deadenylated transcript by 10–25 nucleotides [19,23]. In this way, the LSm1–7–Pat1 complex may prevent the exosome from digesting the mRNA, thus diverting it to the 5′→3′ decay pathway (Figure 1).

**How is Pat1 recruited to mRNAs, and does it affect all mRNAs?**

Many questions remain regarding the manner by which the yeast Pat1p is recruited to mRNA. Pat1p can bind poly(U) RNA homopolymers; in line with the LSm1–7–Pat1 complex RNA binding being enhanced by the presence of a U stretch at the 3′-end [16,19]. In addition, *Xenopus*, as well as human Pat1 proteins, interact with RNA [24–26]; however, there is no evidence as yet to indicate whether Pat1 proteins bind mRNA directly or via protein partners.

Another unresolved issue is whether the yeast Pat1p binds all mRNAs or a specific subclass of mRNAs. A global reduction in protein synthesis is observed when Pat1p is overexpressed [13], indicative of Pat1p acting non-specifically. But whether the endogenous Pat1p acts similarly is unclear. Indeed, it has been reported that Pat1p acts on specific mRNAs; for example, *PABP1* mRNA is not stabilized in a Pat1p-deletion strain [8]. However, this mRNA may be degraded via an alternative decay route. Lastly, the timing of Pat1p recruitment to the mRNP is not known. Several lines of evidence hint at Pat1p being a shuttling protein. First, Pat1p is present in both cytoplasmic and nuclear fractions [11]. Secondly, in an LSm1-deletion strain, Pat1p partially relocalizes to the nucleus [15]. Thirdly, a yeast two-hybrid assay shows Pat1p as an interacting protein of...
Evolution of two Pat1 protein paralogues in vertebrates

Invertebrates and yeast only have one Pat1 protein (Figure 2), which has been characterized as both a translational repressor and an mRNA decay factor [13,24]. In contrast, vertebrates have evolved two Pat1 proteins, e.g. the human Pat1a and an mRNA decay factor [13,24]. In vertebrates, Pat1a and Pat1b proteins have diverged in order to separate their disparate functions, and often display similar decay rates [31–33]. These types of mRNAs are part of so-called ‘decay regulons’ (for a review, see [34]) and may therefore be bound by specific RNA-binding proteins. Could Rpb4 and Rpb7 regulate these related mRNAs by coupling their rate of transcription with their rate of decay [35], and likewise could Pat1p play a similar role?

Evolution of two Pat1 proteins in vertebrates, Pat1a and Pat1b

Invertebrates and yeast only have one Pat1 protein (Figure 2), which has been characterized as both a translational repressor and an mRNA decay factor [13,24]. In contrast, vertebrates have evolved two Pat1 proteins, e.g. the human Pat1a and Pat1b (previously named PatL2 and PatL1) [25,36] whose respective homologues in Xenopus laevis are xPat1a (previously named P100) and xPat1b [26,37–39] (Figure 2). Pat1b proteins are more conserved between each other than Pat1a proteins which show greater variation within their protein sequences. The most conserved region across both Pat1a and Pat1b families lies in their C-terminal half [25,26,36,42]. The evolution of two paralogous proteins in vertebrates naturally raises the question of whether these proteins have diverged in order to separate their disparate functions, and/or to allow for tissue-specific roles. Indeed, Xenopus xPat1a is an oocyte-specific protein, whereas xPat1b is mostly expressed in eggs and embryos. Both xPat1 proteins were demonstrated to repress translation when tethered to reporter mRNA in oocytes [26,37]. Importantly, their role in translational repression in oocytes, rather than mRNA decay, is in line with the observation that decapping activity is only detected at the midblastula transition (in stage 12 embryos), and not before [40,41]. Human Pat1b is present in many somatic tissues, and in tissue culture cells, in which hPat1a is not detected [26,36,42]. It has been shown recently that hPat1b, which localizes to P-bodies, couples mRNA deadenylation with mRNA decapping via multiple sets of interactions [25,42]. In contrast, when mammalian Pat1a is overexpressed in tissue culture cells, it either localizes weakly to P-bodies or has a dominant-negative effect on them [26]. We therefore hypothesize that the maternally expressed Pat1a proteins may be required solely in translational control, whereas the somatic Pat1b proteins mediate mRNA decay. More systematic studies, including careful analysis of binding partners in different cell types and domain swap experiments, should shed light on the respective functions of the two vertebrate Pat1 proteins.

Conserved roles for metazoan Pat1 proteins

Remarkably, since April 2010, altogether seven papers on metazoan Pat1 proteins have been published (see the Note added in proof for an update). Given the timing and space constraints in the present review, we are only able to summarize briefly the main common observations, and to point out any interesting differences and remaining issues to address.

The recently published evidence shows that both the Drosophila (HPat) and human Pat1b are involved in 5′→3′ mRNA decay in tissue culture cells. Indeed, HPat and hPat1b have been demonstrated to link the deadenylation and decapping steps in mRNA decay, using tethering assays alongside knockdowns of Dcp1/Edc4 or overexpression of dominant versions of Caf1 deadenylase and Dcp2, as well as direct decapping assays with immunoprecipitated protein and cap-labelled substrate RNA [24,25,42] (Figure 3).
Post-Transcriptional Control: mRNA Translation, Localization and Turnover

**Figure 3** Cartoon depicting yeast Pat1p, Drosophila HPat and human Pat1b proteins with their region boundaries (not to scale)

Pat1p is divided into five regions (here named I–V for simplicity) [16]. HPat is divided into four regions: N-ter (N-terminal), proline-rich, MID (mid-region) and Pat-C (Pat-C-terminal) regions [24]. HPat1b has been delineated into regions with somewhat different boundaries by two groups. Braun et al. [25] delineated HPat1b in the same way as the Drosophila HPat, whereas Ozgur et al. [42] divided the protein into the A (acidic), N (N-terminal), H (homology) and C (C-terminal) regions. The roles of the various Pat1p regions as well as their associated partners are indicated. The dotted lines indicate the boundaries of similar domains.

In agreement with the functional data, tagged HPat and HPat1b proteins co-immunoprecipitate with other P-body components, including Me31B/p54 RNA helicase, the decapping enzymes and cofactors Dcp1, Dcp2, Edc3, Edc4 (Ge-1, Hedls) and Lsm1–7, components of the Ccr4–Caf1–Pop2–NotI deadenylase complex, and Xrn1, although not all interactions are seen strongly with both Pat1 proteins [24,25,42] (Figure 3). Furthermore, HPat was shown to interact with GW182 [43], a P-body miRNP component. Thus HPat may have an additional role in miRNA function; indeed, it has been estimated by microarray analysis that it regulates 15% of AGO1 (Argonaute 1) mRNA targets [44].

Mapping experiments indicated that the Me31B/p54 RNA helicase interacts with the extreme N-terminal 1–55/70 amino acids in metazoa, whereas the further downstream N-terminal, central and C-terminal regions bind the remaining factors, with mostly minor variations regarding their binding sites and relative affinities [24,25,42]. Overall, at least three separate domains seem to interact with different Pat1 partners, including decapping, deadenylation and exonuclease factors (Figure 3). Given the near coincident publication of several of the papers summarized in Figure 3, and the lack of obvious domains, it is perhaps not surprising that precise boundaries differ between groups. Overall, it has been suggested that Pat1 proteins are scaffold proteins and couple deadenylation and decapping via their multiple interactions [24,25,42]. However, interestingly, we note that, whereas yeast Pat1p and Drosophila HPat are required for decapping, their depletion does not affect deadenylation [8,9,24,44].

Supporting their conserved roles in mRNA decay, Pat1 proteins localize to P-bodies in Drosophila and human cells, shown using transfected fluorescent protein fusions as well as by immunostaining of endogenous proteins [6,24–26,36,42]. P-body residency is also observed in yeast and Caenorhabditis elegans [14–16,45,46]. However, there is not complete agreement regarding whether Pat1 proteins are essential for P-body assembly, possibly arising from experimental and/or cell variation. Lack of yeast Pat1p only appears to have a slight effect on P-body size, but does not lead to their loss [13,14], unlike depletion of HPat in Drosophila S2 cells [6,14]. Moreover, in different human cell lines, Pat1b siRNA (short interfering RNA) can lead to partial or near complete loss of P-bodies [26,42]. There are also interspecies differences regarding which domain(s) target Pat1 proteins to P-bodies. In the case of yeast and human Pat1 proteins, it is the C-terminal region that targets them to P-bodies, whereas the N-terminal portion (downstream of the Me31B/p54 helicase-binding domain) serves this function in Drosophila HPat [16,24,25,42] (Figure 3). Perhaps it would therefore be safe to conclude that Pat1 proteins are not core structural components of P-bodies, but associate with them via dynamically changing interactions, or subtly different interactions in different cell types.

### Decay factors, P-bodies and viruses

Some RNA viruses use their RNA both as genomic and transcript information. In order to prevent competition between these two types of RNAs, viruses may use the host mRNA machinery to separate/compartimentalize them (reviewed in [47]). The mechanisms involved in this separation have not yet been elucidated. Yet, there is increasing evidence that viral particles and proteins localize to P-bodies [47–49]. Decay factors such as Dhh1/p54, the LSm1–7 complex and Pat1 proteins are necessary for efficient translation of viral RNAs and the replication of their genomic RNAs, in the case of Brome mosaic virus in yeast and Hepatitis C virus in human cells [50–52]. Yeast Pat1p is also required for Ty1 retrotransposition [49]. In contrast, we note that, in the case of HIV-1, depleting P-body components such
as p54, Lsm1, Xrn1 and GW182, all Pat1 partners, increases viral production and infectivity [53,54]. Although the role of Pat1 in virus regulation and retrotransposition remains obscure, plausibly in addition to their normal silencing functions, Pat1 and other P-body components may act as activators of expression in some cell contexts. Overall, the key question of why some viruses use these components for their life cycle remains to be addressed.

**Outlook**

In summary, Pat1 proteins act as scaffold or remodelling proteins involved in the transition between actively translating mRNPs to repressed mRNPs, and link deadenylation and decapping by sequentially binding different factors involved in translational repression, deadenylation and decay. A major unresolved issue regarding the functions of Pat1 proteins is their detailed mechanisms of action which eventually leads to mRNA degradation. Another important aspect is to understand why vertebrates have evolved two paralogous Pat1 proteins. Interestingly, maternal and somatic P-body-like RNP (ribonucleoprotein) possess additional components which have differentially expressed paralogues such as ePAB (embryonic PAB)/PABP, Rapa55/B/A and elf4e1a/b [38,55]. The systematic characterization of these protein pairs, including Pat1a/b, would inform on their respective functions in gene regulation during the oocyte to embryo transition, which occurs in the absence of transcription, and in the soma.

**Note added in proof (received 12 October 2010)**

Since submission of the present paper, Nissan et al. [56] have characterized further the scaffold nature of yeast Pat1p in their analysis of decapping activators, and Totaro et al. [57] have described human Pat1b as a deadenylation co-factor.

**Acknowledgements**

We thank Ania Wilczynska, Nicola Minshall and Helen Broomhead for discussions and advice.

**Funding**

We gratefully acknowledge project funding from the Wellcome Trust and Biotechnology and Biological Sciences Research Council (BBSRC) (to N.S.), and the BBSRC for a Ph.D. studentship (to A.M.).

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Received 30 June 2010
doi: 10.1042/BST0381602

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