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An additional class of m⁶A readers

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Abstract

Abundant N⁶-methyladenosine (m⁶A) mRNA-modification influences mRNA fate by stimulating recruitment of m⁶A reader proteins. A previously unappreciated class of m⁶A reader proteins is now shown to use a common RNA-binding domain and flanking regions to selectively bind m⁶A-containing mRNAs increasing their translation and stability.

The base modification N⁶-methyladenosine (m⁶A) is an abundant and dynamic modification in eukaryotic mRNAs. The reversible m⁶A modification of an mRNA molecule can impact every step in its life cycle, including splicing, export, translation, and decay¹. The functions of m⁶A are mediated by RNA binding proteins that selectively bind to m⁶A-containing RNAs, called m⁶A reader proteins. The m⁶A reader proteins characterized to date recognize m⁶A-containing RNAs either by using a YT521-B homology (YTH) domain to directly bind the m⁶A base^{1,2}, or by binding to single-stranded RNA motifs that become exposed upon m⁶A-induced changes in RNA structure^{3,4}. On page xxx of this issue, Huang *et al.*⁵ discover an additional class of m⁶A reader proteins that use K Homology (KH) domains to selectively recognize m⁶A-containing RNAs and promote their translation and stability.

The YTH domain family of proteins has been proposed to sort m⁶A-containing transcripts into a fast track with accelerated nuclear export, translation, and decay⁶. One of the well-established functions of m⁶A modification is to promote mRNA decay through a mechanism mediated by the m⁶A reader protein YTH domain family 2 (YTHDF2)⁷. Consistent with the association between m⁶A and mRNA decay, knockout of an m⁶A methyltransferase leads to a global decrease in m⁶A levels as well as an increase in the steady-state levels of mRNAs that contain m⁶A in the wild-type cells⁸. However, overexpression of an m⁶A demethylase reduces both the m⁶A content and the steady-state levels of hundreds of transcripts, and some of these transcripts have been shown to be more stable in the presence of m⁶A modification⁹. These results raise the possibility that m⁶A reader proteins other than YTHDF2 influence the stability of a subset of m⁶A-containing transcripts.

Huang *et al.*⁵ report that insulin-like growth factor-2 mRNA binding proteins 1, 2, and 3 (IGF2BP1–3) are m⁶A reader proteins that stabilize their m⁶A-containing target transcripts.

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IGF2BP1–3 were enriched in pull-downs using an m⁶A-containing single-stranded RNA bait, and m⁶A was enriched in RNA species that immunoprecipitated with IGF2BP1–3. Moreover, about 80% of IGF2BP target transcripts contained m⁶A, and the IGF2BP consensus binding site was UGGAC, which overlaps with the m⁶A consensus sequence DRACH (D = A/G/U, R = A/G, H = A/C/U)¹⁰. IGF2BP targets tended to have higher transcript levels and longer half-lives than non-target transcripts, whereas YTHDF2 targets had shorter half-lives than non-target transcripts. Knocking down individual IGF2BPs or m⁶A methyltransferase led to decreased steady-state levels of IGF2BP-bound transcripts. In addition, IGF2BP3 knockdown shortened the median half-life of its target mRNAs, and IGF2BP1–2 or m⁶A methyltransferase knockdown also shortened the half-lives of several IGF2BP-bound transcripts. Thus, the m⁶A reader proteins IGF2BP1–3 could be responsible for the stabilization seen in the subset of m⁶A-containing transcripts following m⁶A modification⁹.

To further investigate the mechanisms by which IGF2BP1–3 stabilize m⁶A-containing transcripts, Huang *et al.*⁵ searched for IGF2BP-interacting proteins that enhance mRNA stability. Several proteins known to stabilize mRNA also interacted with IGF2BP1–3. One of these proteins, Hu antigen R (HuR), co-localized with IGF2BPs in P-bodies, suggesting that IGF2BPs and HuR work together to protect mRNAs from degradation. Following heat shock, IGF2BPs localized to stress granules, possibly to store and stabilize mRNAs during stress. In addition, IGF2BP1–3 and HuR were present in polysome fractions, and IGF2BP1 played a role in recruiting its m⁶A-containing target mRNA *MYC* to polysomes. The authors also constructed a luciferase reporter with an m⁶A-containing region of *MYC* mRNA that is critical for IGF2BP binding, called the coding region instability determinant (CRD). IGF2BP1–3 promoted expression of the CRD-containing reporter in an m⁶A-dependent manner. Both the polysome fractionation and luciferase reporter data suggest that IGF2BP1–3 could promote translation of their m⁶A-containing target transcripts. Therefore, in contrast to the fast-track pathway coordinated by the YTH domain family of proteins⁶, the IGF2BPs seem to direct m⁶A-containing transcripts into an alternative pathway with increased translation, storage, and stability.

The m⁶A reader proteins identified to date fall into two classes, sorted based on their mechanisms for selective binding to m⁶A-containing RNAs. Class I includes the m⁶A reader proteins that contain a YTH domain. In these m⁶A readers, the YTH domain directly and selectively binds the m⁶A base in a hydrophobic aromatic cage¹ (Fig. 1a). Class II includes two m⁶A reader proteins so far, both of which are heterogeneous nuclear ribonucleoproteins (hnRNPs). These m⁶A readers selectively bind m⁶A-containing RNAs through an m⁶A-switch mechanism, in which m⁶A decreases the stability of Watson-Crick base-pairing and thereby increases the accessibility of a single-stranded RNA binding motif that is recognized by the m⁶A reader^{3,4} (Fig. 1b).

The m⁶A readers IGF2BP1–3 lack a YTH domain and bind to m⁶A-containing RNA independently of RNA structure, suggesting that they use a third mechanism to recognize m⁶A-modified transcripts. The IGF2BPs are composed of two RNA recognition motifs (RRMs) and four KH domains. By truncating RRM or mutating KH domains, Huang *et al.*⁵ determined that only the third and fourth KH domains (KH3–4) were required for the

selective recognition of m⁶A-containing RNAs. Moreover, mutations in KH3–4 reduced IGF2BP binding to the m⁶A-containing *MYC* CRD and abolished the m⁶A-dependent promotion of luciferase reporter expression by IGF2BP2. Whereas the m⁶A-selective YTH domains are only present in five human proteins¹, KH domains are among the most common RNA binding domains, found in dozens of human RNA binding proteins¹¹. It is surprising that the common KH domains could be responsible for the selective binding of IGF2BP1–3 to m⁶A-containing transcripts in an RNA-structure-independent manner. In fact, the KH3–4 di-domain alone was insufficient for selective binding to m⁶A-containing RNAs; the authors suggested that regions flanking the KH domains could also contribute to m⁶A selectivity. Thus, while the exact mechanisms of m⁶A selectivity have yet to be worked out, IGF2BP1–3 establish a third class of m⁶A reader proteins, class III, in which common RNA binding domains and their flanking regions work together to selectively bind m⁶A-containing transcripts (Fig. 1c). The m⁶A reader protein hnRNPA2B1, which also lacks a YTH domain, could fall into either class II or III. One of the RRM of hnRNPA2B1 might partly account for its m⁶A selectivity, possibly by acting in combination with flanking regions^{12,13}. Since RRMs are common RNA binding domains¹¹, this mechanism may define hnRNPA2B1 as another class III m⁶A reader protein.

In addition to identification and characterization of the m⁶A reader proteins IGF2BP1–3, Huang *et al.*⁵ examined the role of m⁶A selectivity in the oncogenic functions of IGF2BPs. The authors found that IGF2BP1–3, as well as their target mRNA *MYC*, were amplified or overexpressed in multiple types of cancer. IGF2BP1–3 knockdown or knockout decreased *MYC* protein expression, cell proliferation, and colony formation in human cancer cell lines. The cell proliferation and colony formation phenotypes of the knockout cell lines could be rescued by wild-type IGF2BPs, but not by KH3–4 mutants. Therefore, m⁶A selectivity is required not only for IGF2BPs to promote mRNA translation and stability, but also for the oncogenic functions of IGF2BP1–3.

The m⁶A readers IGF2BP1–3 discovered by Huang *et al.*⁵ present an alternative pathway by which m⁶A affects mRNA stability and establish a third class of m⁶A reader proteins that use common RNA binding domains and their flanking regions to bind m⁶A-containing RNAs. The contrasting effects of YTHDF2 and IGF2BP1–3 on the stability of their m⁶A-containing targets raise several questions: do YTHDF2 and IGF2BPs compete for the same target mRNAs, and which features of an m⁶A-containing mRNA determine whether it is targeted for decay or stabilization? In addition, the role of KH domains in the selective binding of m⁶A-containing RNAs is intriguing, but the mechanisms by which such common RNA binding domains can specifically recognize m⁶A-containing transcripts have yet to be elucidated. The work presented in this study expands the known knowledge of m⁶A reader protein mechanisms and functions, while raising questions about how these elements fit into the broader network of m⁶A-dependent cellular pathways.

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Figure 1.

Three classes of m⁶A reader proteins. Class I m⁶A reader proteins use a YTH domain (blue) to directly bind the m⁶A base (red). This class includes the five human proteins that contain a YTH domain. Class II m⁶A reader proteins use an m⁶A-switch mechanism to bind m⁶A-containing transcripts: m⁶A modification of RNA destabilizes Watson-Crick base-pairing and increases the accessibility of a single-stranded RNA motif (cyan), which is recognized by the m⁶A reader protein (green). The RNA motif can also overlap with the m⁶A site (not shown for simplicity). This class includes hnRNPC, hnRNPG, and possibly hnRNPA2B1. Class III m⁶A reader proteins use a common RNA binding domain (RBD) and its flanking regions (green) to recognize m⁶A-containing transcripts. This class includes the IGF2BPs, which use KH domains and their flanking regions to selectively bind m⁶A-containing RNAs, and possibly hnRNPA2B1, in which the RRM and its flanking regions might contribute to m⁶A selectivity.