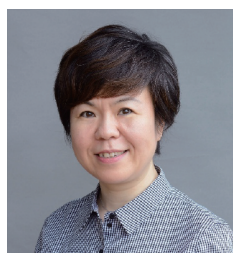


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程红,中国科学院分子细胞科学卓越创新中心(原上海生物化学与细胞生物学研究所)研究组长、研究员、博士生导师,中国科学院“百人计划”和上海市“浦江人才计划”获得者。1998年毕业于中国医科大学,获医学学士学位;2003年毕业于日本神户大学,获博士学位。2003年至2008年,在美国哈佛大学医学院细胞生物学系从事博士后研究。2008年回国,担任中国科学院上海生物化学与细胞生物学研究所研究员。主要从事RNA转运与降解的机制和功能研究,旨在揭示新生RNA出核转运或核内降解命运决定的机制、调控和生物学意义,重点研究方向包括:mRNA出核与pre-mRNA加工的复杂关联、RNA的质量控制和核内降解及其调控、细胞亚核结构调控RNA出核或降解的生理病理机制。自2008年独立组建以来,实验室取得了一系列前沿性进展,先后在*Mol Cell*、*Nat Commun*、*Genes Dev*、*EMBO J*、*PNAS*、*J Cell Biol*、*Nucleic Acids Res*等国际知名学术期刊上发表多篇具有重要影响力的论文。近期发表的论文被F1000Prime推荐,获生物通和BioArt等专评,并被国际知名期刊*Nat Rev Genet*等正面引用和评述。

内含子保留与3'非翻译区延长介导的信使RNA核滞留

蒋清怡^{1,2}, 程红^{1,3*}

(1 中国科学院分子细胞科学卓越创新中心,上海 200031;2 中国科学院大学,北京 100049;3 国科大杭州高等研究院生命与健康科学学院,杭州 310024)

摘要: 在真核细胞中,信使RNA(messenger RNA, mRNA)的出核转运是基因表达的关键步骤。新生mRNA前体只有在细胞核内完成剪接、3'端加工等步骤后才能被运出核,在细胞质中被翻译成蛋白质。mRNA加工过程往往会影响到其出核转运,其中内含子保留(intron retention, IR)及3'非翻译区(untranslated region, UTR)延长是导致mRNA核滞留的重要因素。本综述阐述了两者的介导mRNA核滞留的分子机制及其相关生理、病理功能,并探讨了两者的协同调控,以期理解mRNA核滞留如何调控基因表达提供见解。

关键词: 内含子保留;3'非翻译区;可变加工;核滞留;mRNA出核转运

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Intron retention and 3' UTR lengthening mediate mRNA nuclear retention

JIANG Qing-Yi^{1, 2}, CHENG Hong^{1, 3*}

(1 Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences, Shanghai 200031, China; 2 University of Chinese Academy of Sciences, Beijing 100049, China; 3 School of Life Science, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou 310024, China)

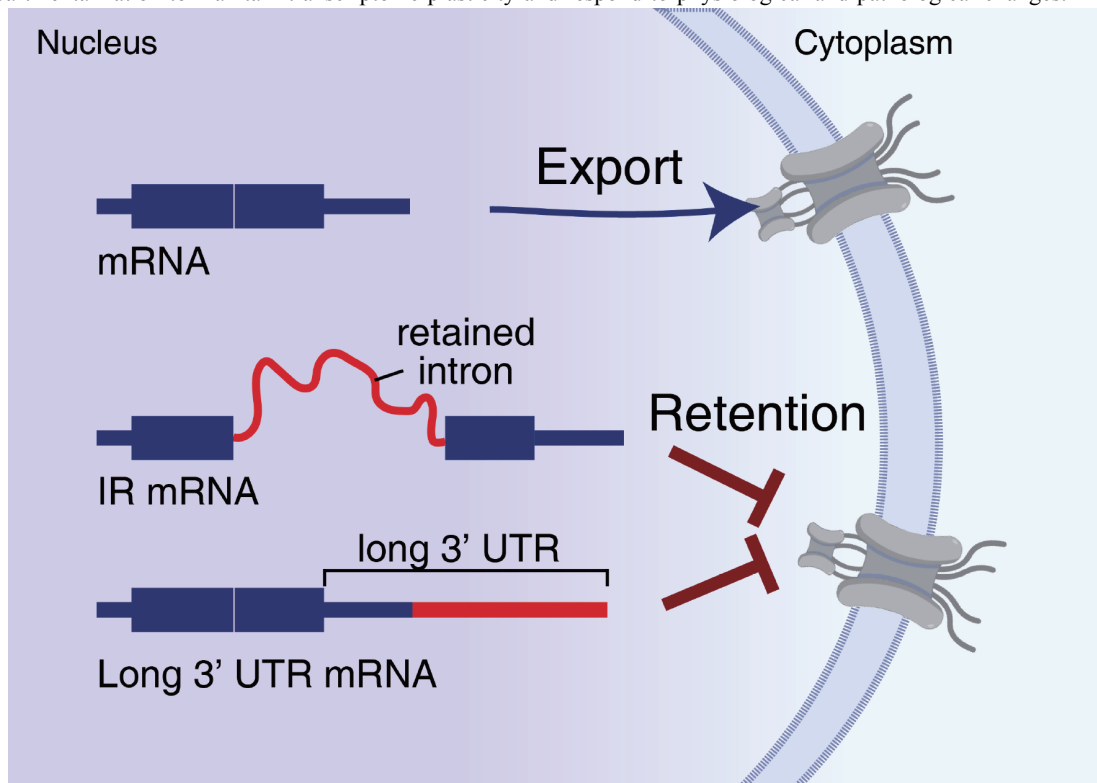
Abstract: In eukaryotic cells, the export of messenger RNA from the nucleus to the cytoplasm is a critical step in gene expression. This process is tightly linked to pre-mRNA processing, such as splicing and 3' end maturation, which act as checkpoints for export. While most mRNAs are exported rapidly after processing, certain transcripts are held back in the nucleus, a phenomenon known as mRNA nuclear retention. Once thought to be a side-effect in RNA processing, nuclear

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*通信作者:E-mail: hcheng@sibcb.ac.cn

retention is now recognized as a deliberate and sophisticated gene regulatory mechanism. Two major factors driving this process are intron retention and the lengthening of 3' UTR. This review explores how these two events determine mRNA nuclear retention. First, current research underscores introns as strong gatekeepers of mRNA nuclear export. Specifically, retained introns prevent mRNAs from leaving the nucleus. These transcripts are either degraded by nuclear quality control or stored as a stable “nuclear reservoir”. When the cell encounters stress or external stimuli, these retained RNAs can be rapidly spliced and exported to produce proteins. This allows the cell to respond much faster than by starting new transcription. However, if this retention fails, escaped IR transcripts can lead to the production of abnormal proteins, contributing to diseases like cancer and neurodegeneration. Beyond introns, long 3' UTRs produced from alternative polyadenylation (APA) provide additional docking sites for retention factors. Compared to shorter versions, long 3' UTRs often contain specific sequences or structures that promote nuclear retention. These elements recruit protein factors that anchor the mRNA within nuclear bodies such as paraspeckles. On the other hand, chemical modifications on long 3' UTRs can limitedly buffer this retention by dynamically regulating the binding of export factors. Finally, we discuss how intron retention and 3' UTR lengthening can cooperate to enhance the nuclear retention of mRNAs. Understanding such mechanisms will reveal how cells use nuclear compartmentalization to maintain transcriptome plasticity and respond to physiological and pathological changes.



Key words: intron retention; 3' UTR; alternative processing; nuclear retention; mRNA export

在真核细胞中,核膜(nuclear envelope)将作为遗传物质的DNA分隔在细胞核内,而蛋白质合成发生在细胞质中。这种空间上的区室化,决定了DNA转录产生的mRNA必须从细胞核转运至细胞质中,才有机会被翻译产生蛋白质,进而发挥生物学功能^[1]。mRNA出核过程受到严密的监控,新合成的mRNA前体只有在完成剪接、3'端加工等步骤后,才能有效招募出核因子,获得出核能力^[2-4]。mRNA加工形式的变化,可以直接影响成熟mRNA的出核转运。其中,可变加工造成的内含子保留和3' UTR的延长是导致mRNA核滞留的两个重要因素。mRNA

核滞留作为基因表达调控的新层面,虽曾长期被忽视,但近年正逐渐被认为是一种重要的转录后调控机制。研究内含子保留和3' UTR延长引起mRNA核滞留的机制,并解析其在重要生理病理过程中的功能,对深入理解真核基因表达调控具有重要意义。

1 mRNA的剪接与3'端加工

剪接和3'端加尾是mRNA前体加工的两个重要过程。简单来说,剪接过程将mRNA前体中的内含子去除,并将外显子连接起来,形成连续的mRNA序列^[5]。对于绝大多数mRNA而言,其3'端加工过

程包括切割与在上游切割产物的3'端加上多聚腺苷酸尾两个步骤^[5,6]。这些加工步骤的完成是mRNA获得出核许可的前提^[7-9]。

mRNA的加工方式并非单一不变,同一基因可以产生不同的加工变体,这些变体往往具备不同的出核能力^[5,10]。可变剪接(alternative splicing, AS)和可变加尾(alternative polyadenylation, APA)是mRNA可变加工的两种重要方式。随着高通量测序技术的进步,现已证实超过95%的高等真核编码基因会发生不同程度的可变剪接^[11,12];其中,内含子保留(intron retention, IR)作为可变剪接的一种重要类型,会导致mRNA中携带内含子序列。近年研究表明,发生IR的转录本有两种不同的命运:一部分转录本中的保留内含子不能被剪接机器有效地识别,此类转录本可被正常运输出核;然而,更多的IR转录本被识别为滞留内含子转录本(detained intron RNA, DI-RNA),其携带的内含子导致mRNA滞留在细胞核中^[13-15]。鉴于后者极强的核滞留特性,本文后续讨论的IR转录本均为DI-RNA类型。

可变加尾同样是一种被广泛使用的加工机制,据统计,超过70%的高等真核编码基因会受到可变加尾的调控^[6,10]。可变加尾通过使用基因上不同位置的加尾信号(polyadenylation site, PAS),产生不同的可变加尾异构体。可变加尾多数发生在3' UTR上,导致不同异构体具有不同长度的3' UTR:近端加尾信号的使用产生较短的3' UTR,而远端加尾信号的使用则导致了3' UTR的延伸^[16]。多项研究表明,长3' UTR往往与较低的mRNA出核效率相关,提示其是一种重要的核滞留信号^[17-19]。

由此可见,mRNA前体的可变加工不仅改变了转录本的序列组成,也会影响mRNA的出核效率,甚至造成mRNA核滞留。解析引起mRNA核滞留的机制,有助于理解mRNA出核在基因表达精准调控中的功能,并为理解相关生理病理过程提供重要线索。

2 mRNA出核转运与核滞留

mRNA出核转运由多种出核因子协同完成,其中在进化上高度保守的转录-输出复合体(transcription-export, TREX)与核RNA输出因子1-核运输因子2样输出因子1(nuclear RNA export factor 1-nuclear transport factor 2 like export factor 1, NXF1-NXT1)出核受体在该过程中发挥核心作用^[8,20]。NXF1-

NXT1异二聚体通过与核孔蛋白(nucleoporin, Nup)相互作用,帮助mRNA穿过核孔复合体(nuclear pore complex, NPC)^[3,21]。然而,由于NXF1-NXT1自身与mRNA的结合能力较弱,它与mRNA的结合往往需要接头因子来介导。TREX复合体的组分ALYREF是最常见的mRNA出核接头因子^[20,22],其招募伴随转录进行,并依赖TREX的另外两个组分THO亚复合体和UAP56^[8,20,23]。只有当TREX各组分装配完成、NXF1-NXT1稳定结合后,mRNA才能经过核孔复合体,被输出到细胞质中^[8,22,24,25]。

然而,出核因子的成功招募并非mRNA出核的充分条件,待出核的转录本还必须摆脱核滞留信号的限制。mRNA在细胞核与细胞质之间的分布,本质上是出核转运与核滞留两种信号的动态平衡。关于mRNA出核转运命运的决定,目前领域内存在两种观点:出核优先(export-first)模型认为,完成加工的mRNA倾向于自发结合出核因子,核滞留是通过特定机制主动拦截的结果^[7-9]。与之相对的滞留优先(retention-first)模型则提出,新生mRNA倾向于滞留在细胞核内,只有成功招募足够的出核因子才能获得出核许可,而核滞留本质上是mRNA加工未完成、缺乏足够许可的表现^[25-27]。值得注意的是,无论基于何种模型,mRNA的出核转运调控均可被视为出核许可和滞留之间的对抗。主要形式的mRNA能够快速出核转运,而携带内含子或长3' UTR的mRNA,则因为较强的滞留阻力干扰出核动力,最终导致出核受阻。

这种核滞留可以通过多种分子机制实现,进而使被滞留的mRNA呈现出不同的空间分布特征。首先,带有特定基序或碱基修饰的mRNA,在转录完成后可持续保持与染色质的结合状态^[28,29],导致其被限制在染色质上。其次,含有内含子或非经典剪接位点的mRNA,往往会被剪接相关因子识别,并限制在核斑(nuclear speckles, NS)这种无膜细胞核区室中^[30,31]。此外,部分具有特殊二级结构元件的长3' UTR转录本可以招募特定的RNA结合蛋白,进而将mRNA扣留在核旁斑(paraspeckles)中^[17]。

综上所述,细胞通过识别mRNA上的特定信号,利用不同机制将其限制在不同的核内空间,最终实现mRNA的核滞留。基于此,本综述将重点梳理内含子保留与3' UTR延长调控mRNA核滞留的分子机制,并探讨细胞中mRNA核滞留调控的生物

学意义。

3 内含子保留调控mRNA核滞留

3.1 可变剪接与内含子保留

剪接是由剪接体(spliceosome)介导的内含子剔除和外显子连接过程。剪接体以U1、U2、U4/U6、U5五种小核核糖核蛋白(small nuclear ribonucleoprotein particles, snRNP)为核心,同时包含十九号复合物(nineteen complex, NTC/Prp19)及其相关的NTR(NTC related)复合物等亚结构,并在SR蛋白、异质核糖核蛋白(heterogeneous ribonucleoprotein particle, hnRNP)家族等多种调节因子的辅助下,形成高度动态的分子机器^[32]。在剪接过程中,U1小核核糖核蛋白与U2小核RNA辅助因子(U2 small nuclear RNA auxiliary factor, U2AF)二聚体首先分别识别5'和3'剪接位点(splicing site, SS),进而招募U2小核核糖核蛋白至分支点(branch point, BP),并使该位点的腺苷酸完成构象活化^[32]。随后,经由两步转酯反应,内含子以套索形式被切除,相邻外显子则实现连接^[32]。剪接的高效完成是mRNA加工成熟并获得出核能力的关键之一。

然而,转录本的剪接模式并不单一。内含子保留作为可变剪接的一种重要类型,可在超过70%的蛋白质编码基因中发生^[2,33-35]。对单个基因而言,内含子保留事件的产生依赖于较弱的剪接信号:5'剪接位点、3'剪接位点或分支点保守性降低时,剪接体招募和识别的效率就会下降,导致内含子无法被切除^[5,14,36,37]。这类内含子通常具有显著的序列特征:长度相对较短,GC含量较高,且倾向于位于转录本的3'端;此外,两侧外显子的GC含量通常也更高,而内含子本身及其上游外显子的长度则相对更长^[14]。

由于IR转录本的产生来源于剪接效率的降低,它曾一度被视作mRNA加工不完全的产物,在核内产生后发生降解^[38,39]。因此,其核滞留也被认为是RNA质量监控的重要机制:防止还未及时降解的IR转录本进入细胞质,翻译产生截短形式的蛋白质,影响正常的生理过程^[33,40]。然而,研究发现,事实上IR转录本可以稳定存在于细胞核内,并在细胞响应特定信号后,启动保留内含子的剪接,使细胞能够在无需重启转录的情况下,快速释放特定mRNA出核,即时响应表达需求^[13-15]。这一发现揭示了由内含子保留引起的mRNA核滞留在基因表达中的积极作

用,IR转录本也因此成为解析mRNA核滞留机制的重要模型。

3.2 内含子保留介导mRNA核滞留的机制

前文已经提到,mRNA的出核效率与其加工状态紧密相关^[8]。mRNA经过剪接后,外显子连接处组装的外显子拼接复合体(exon-exon junction complex, EJC)作为招募出核因子的重要支架,驱动mRNA向胞质转运^[41]。然而,IR转录本的核滞留现象存在明显的矛盾之处:即便转录本已剪接的区域招募了EJC和出核因子,其残留的内含子序列仍可构成极强的核滞留信号,限制mRNA出核转运^[8,42]。进一步的亚细胞定位观察发现,这些核滞留的mRNA主要聚集于染色质、核斑等与转录活动密切相关的亚核区域^[30,43,44],提示它们在转录产生后的早期阶段已受到出核限制。

机制研究发现,剪接因子对于内含子的识别和扣留,是导致IR转录本核滞留的关键^[45,46]。1989年在酵母体系中进行的研究发现,突变滞留内含子的剪接位点或分支位点可导致IR转录本迅速从核内释放进入胞质^[45,46]。这表明,IR转录本的核滞留主要依赖于剪接体对内含子的识别,而不仅仅由内含子序列本身决定。作为剪接体十九号复合物的核心组分之一,歪颈mRNA前体剪接因子1(crooked neck pre-mRNA splicing factor 1, CRNKL1)在这种识别中发挥了重要作用^[47]。CRNKL1通过稳定剪接体的组装与构象,增强剪接体对内含子的识别和锚定能力,从而促进由剪接体识别介导的mRNA核滞留(图1)^[47]。

这种依赖蛋白质识别实现的核滞留,本质上是一种分子水平的主动拦截过程。因此,当剪接因子功能受损时,原本被扣留的内含子无法被有效地识别,进而使IR转录本“泄露”进入胞质(图1)^[48,49]。例如,在细胞中抑制核心剪接体组分U2或SR蛋白,均可导致IR转录本在细胞质中的水平显著升高^[34,50,51]。类似地,在外源过表达体系中,带有内含子的mRNA前体转录本也会逃逸至细胞质中并发生翻译^[46]。

在核膜附近同样存在IR转录本的核滞留机制。有研究显示,酵母核孔复合体组分蛋白Mlp1(myosin-like protein 1)与同源的拟南芥NUA(nuclear pore anchor)蛋白能够识别带有内含子的mRNA前体,并将其扣留在核内,限制其出核转

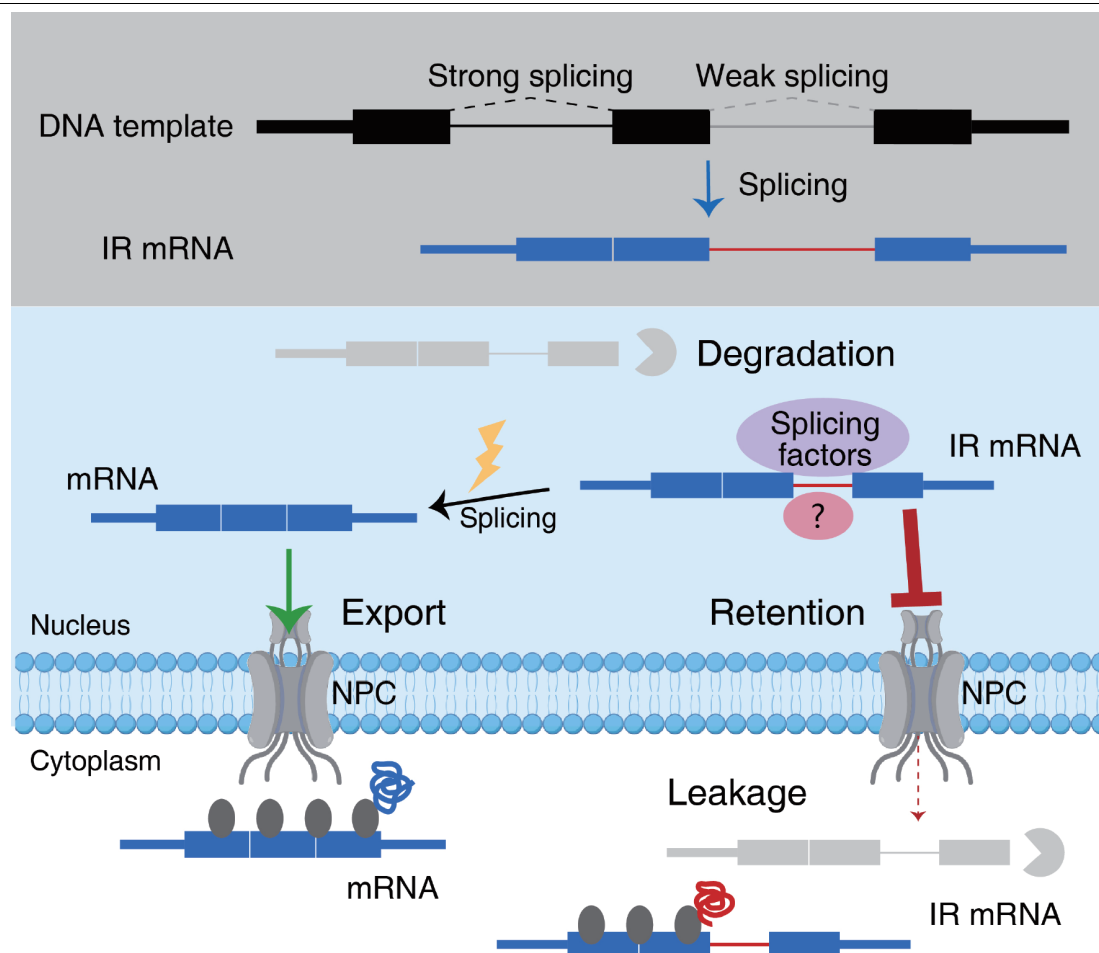


图1 内含子保留调控mRNA核滞留

IR转录本在转录产生后,既可能被核内质量监控机器扣留并降解,也可以作为IR转录本稳定存在。这些IR转录本由于剪接因子的识别而滞留在核内,作为响应刺激的转录本储备。当核滞留失效时,泄露的IR转录本在细胞质中通常受到RNA质控机器的监控,也可在特定生理病理状态下产生新的蛋白变体,发挥新的功能。

Figure 1 The nuclear retention of mRNA regulated by intron retention

After transcription, IR transcripts can either be sequestered by the nuclear quality control machinery for degradation, or persist as stable IR RNAs. These transcripts are retained in the nucleus by the recognition of splicing factors, serving as a reservoir of transcripts responsive to stimuli. When nuclear retention fails, IR RNAs in the cytoplasm can be typically monitored and degraded by NMD; However, under specific physiological or pathological conditions, they may also generate novel protein variants with distinct functions.

运^[52,53]。由此启发的后续研究也发现,人类中同源的核孔复合体组分TPR(translocated promoter region)也能够阻止IR转录本出核^[54]。然而,近年的研究又挑战了这一结论,并提出TPR实际上发挥了促进mRNA出核的作用,其缺失会使得短基因的mRNA出核受到抑制^[55]。上述结论的冲突反映出mRNA核滞留调控的复杂性,也提示NPC及相关蛋白在该过程中的调控作用仍有待进一步阐明。

剪接因子不仅在IR转录本的核滞留中发挥关键作用,还负责招募核内降解机器对核滞留的转录本

进行降解,将核滞留与核内RNA质量监控联系起来^[38,39]。剪接辅助因子U2AF65(U2 small nuclear ribonucleoprotein auxiliary factor 65)可以介导IR转录本上核内外切体(exosome)的招募,使部分IR转录本发生降解^[56]。最新研究发现,出核抑制复合体(repressor of export, REX)成员LENG8(leukocyte receptor cluster member 8)也可能参与IR转录本的核滞留与核内降解过程^[57]。尽管IR的核滞留与降解的具体调控规律与分子机制仍有待深入阐明,但两者的相关性提示,IR转录本的核滞留是细胞主动

调控基因表达的重要手段。

综上所述,内含子保留介导的核滞留是决定mRNA命运的关键调控机制。核滞留的状态为转录本创造了一个调控平台:它一方面通过物理拦截为核内质控系统提供了窗口,确保未加工完成的转录本可以被高效清除;另一方面,核滞留又将IR转录本转化为灵活的转录本储备,使其从单纯的加工副产物转变为可随时调用的基因表达资源。这种机制极大地提升了细胞在转录后水平动态调节基因表达的能力。

3.3 内含子保留介导mRNA核滞留的生理与病理意义

IR转录本广泛存在于不同物种、组织类型及发育阶段中,且表现出显著的差异^[12,35,58]。例如,在神经和免疫系统中IR事件比例最高,而在肌肉组织中则比例最低^[59,60]。这一分布特征提示,核滞留的IR转录本并非仅是剪接效率低下产生的副产品,更有可能发挥了调控基因特异性表达和响应环境变化的重要作用。

功能研究进一步表明,核滞留的IR转录本可以形成重要的转录本储备库。在面对环境变化时,细胞可以利用这些已经完成了转录和前期加工的转录本,仅通过启动单一剪接事件,就可快速产生成熟的mRNA,并将其释放到细胞质中进行翻译,响应基因表达需求,无须从头开始耗时的转录和加工过程(图1)^[13,51,61]。在人胚胎干细胞中,存在成千上万保守且高丰度的IR转录本;当细胞遭受DNA损伤或压力应激(如CDC样激酶被抑制)时,丝氨酸/精氨酸富集剪接因子4(*serine/arginine-rich splicing factor 4*, *Srsf4*)磷酸化水平显著升高,导致剪接因子*Srsf5*(*serine/arginine-rich splicing factor 5*)、*Srsf7*等IR转录本中的内含子快速剪接,促使mRNA出核并进行翻译,实现了快速的分子响应^[13]。在神经系统中,IR转录本也作为转录本储备响应外界刺激:当外界信号与N-甲基-D-天冬氨酸受体(N-methyl-D-aspartate receptors, NMDAR)结合并由钙离子/钙调蛋白依赖激酶(Ca^{2+} /calmodulin-dependent protein kinases, CaMK)通路传入细胞内时,剪接体高效识别并切除*Cdk1*(CDC like kinase 1)等神经信号传递相关基因的滞留内含子,从而快速驱动成熟mRNA的产生、出核与翻译,激活神经反应^[62]。除此之外,多项研究表明,这一储备机制在精子发生与突触可

塑性等过程中也同样发挥重要作用^[58,62]。

此外,IR介导的核滞留还可以作为一种动态开关,参与调控基因的组织特异性表达,从而实现对特定基因表达的下调或沉默。例如,在粒细胞生成过程中,剪接因子U1和U2 snRNP亚基表达水平显著下调,使剪接效率广泛降低,IR事件大量增加^[51];这不仅导致大部分mRNA滞留在核内,还增加了转录本被降解的可能性,从而在多个水平抑制如核纤层蛋白(lamin B1, *Lmnb1*)等关键蛋白的表达,最终完成粒细胞成熟过程中核形态的变化^[51]。这种IR介导的核滞留,使得细胞能够通过核内多维度的转录后调控手段,有效抑制关键蛋白的表达,从而推动细胞分化进程。

这种mRNA核滞留调控状态一旦被破坏,可引发一系列不良后果。衰老和神经退行性疾病的发展往往伴随IR转录本表达谱的改变,提示原有细胞稳态被破坏^[63-65]。不仅如此,这些失调IR转录本的核滞留机制也可能失效,导致这些mRNA逃逸至细胞质中,产生截短蛋白,干扰正常细胞功能。正常情况下,进入胞质的IR转录本由于携带提前终止密码子(premature stop codon, PTC),会被无义介导的降解(nonsense-mediated decay, NMD)途径识别并清除^[66,67]。但在肿瘤等病理环境中,这类质控机制往往被抑制,导致逃逸的IR转录本进一步在细胞质中累积;这些mRNA在编码区域引入了源自内含子的新序列,可能使它们编码新的蛋白异构体,从而增强肿瘤适应性(图1)^[68-70]。

以上研究表明,内含子保留介导的mRNA核滞留是实现基因表达时空特异性调控的重要机制,在生理稳态维持与疾病发生发展中具有重要意义。

4 3' UTR延长动态调控mRNA核滞留

4.1 mRNA的3'端加工与3' UTR长度的动态调控

mRNA的3'端加工主要是由3'端切割加尾(cleavage and polyadenylation, CPA)复合体介导完成。CPA复合体在进化上高度保守,包括切割与多聚腺苷酸化特异性因子(cleavage and polyadenylation specific factors, CPSF)、切割刺激因子(cleavage stimulating factors, CstF)、切割因子I(cleavage factor I, CF I)、切割因子II(cleavage factor II, CF II)以及多种辅助因子。这些组分协同识别mRNA上的PAS,在特定位置对转录本的3'末端进行切割。随

后,多聚腺苷酸聚合酶(poly-A polymerase, PAP)在切割后的3'末端催化合成多聚腺苷酸尾^[71,72]。

如前文所述,同一基因可通过选择性使用不同的PAS位点,产生具有不同长度3' UTR的可变加尾变体^[10]。大量研究表明,3' UTR的可变加工具有一定的规律性:在快速增殖和应激条件下(如mTOR通路激活、DNA损伤和病毒感染时),近端PAS使用增多,导致mRNA的3' UTR普遍缩短^[6,73-75];而在细胞分化、免疫活化及神经元成熟过程中,远端PAS的使用显著增加,使mRNA的3' UTR整体延长^[76-78]。3' UTR上往往存在调控mRNA定位、稳定性、翻译效率等的结构或序列,因此可变加尾引起的3' UTR长度变化可从多个层面调控基因表达^[16]。

4.2 3' UTR延长调控mRNA核滞留的分子机制

多项研究表明,mRNA的出核效率与其3' UTR长度之间呈现负相关^[17,21,79]。人为延长报告基因的3' UTR,可以增加mRNA在细胞核中的积累^[80];对于特定mRNA,当其长3' UTR被切割缩短后,mRNA便可被转运出核^[81,82]。这些结果提示,3' UTR的长度本身可改变mRNA的出核效率。值得注意的是,3' UTR引发的核滞留现象与内含子保留引起的完全核滞留不同,其通常表现为出核效率降低,即mRNA仍可输出至细胞质,但过程明显缓慢^[21,83]。从亚核定位来看,发生核滞留的长3' UTR转录本倾向于富集在染色质区域、核斑及核旁斑等与转录活动密切相关的亚核区室中^[17,19,84],暗示它们可能处于加工尚未完成的“等待状态”。基于上述现象,研究人员提出了“渐进式加尾(sequential polyadenylation)”的加工模型——3' UTR延长介导的核滞留可为mRNA提供一个核内“停留窗口”,使其在核内得以继续加工,并利用序列中潜在的近端PAS位点,进一步缩短3' UTR长度,直至mRNA达到合适状态后再被释放出核^[19,81,82](图2)。然而,在细胞核中,3' UTR如何被识别,又是怎样抑制mRNA出核转运的,目前仍是未解之谜。

3' UTR上通常包含丰富的顺式调控元件,其中包括核滞留信号序列(图2)。例如,反向Alu重复序列(inverted Alu repeats, IRAlus)是3' UTR上最具代表性的核滞留元件,它能够形成茎环结构,经腺苷脱氨酶(adenosine deaminase acting on RNA, ADAR)介导发生腺苷-肌苷碱基编辑(adenosine-to-inosine, A-to-I editing)后,特异性结合多功能核蛋白NONO/

p54^{nb}(non-POU-domain-containing octamer binding protein),将mRNA锚定在核旁斑中^[17,85]。此外,长3' UTR上的C-rich短序列基序可以结合hnRNP K和hnRNP H等蛋白,借助蛋白的核锚定特性,抑制mRNA的出核^[18,86]。还有研究发现,一些mRNA的3' UTR中包含非经典5'剪接位点基序,可被剪接因子U1识别并招募细胞核RNA质控蛋白ZFC3H1(zinc finger C3H1-type containing protein 1),从而引发它们的核滞留和降解^[31,87]。在这些情况下,3' UTR延长介导的核滞留是由RNA结合蛋白介导的主动扣留过程。

尽管长3' UTR总体上是mRNA出核的不利因素,但一些mRNA的长3' UTR由于含有CFI结合基序,可招募出核受体NXF1克服核滞留的影响^[21]。此外,长3' UTR的序列中还携带多种修饰位点,可以通过调控出核相关蛋白的招募,改变mRNA携带的出核驱动力。*N*⁶-甲基腺苷(*N*⁶-methyadenosine, m⁶A)作为真核生物中丰度最高的RNA修饰,在3' UTR区域高度富集^[19,88]。核内m⁶A阅读器YTHDC1可结合m⁶A并招募出核因子,进而促进长3' UTR转录本的出核;而当YTHDC1功能受损时,mRNA的核滞留水平显著升高;不仅如此,RNA结合蛋白FMRP对m⁶A的识别也可以提高mRNA出核水平^[89,90]。与此一致的是,当m⁶A去甲基化酶ALKBH5的表达被抑制时,总mRNA也会表现为出核促进^[91-93]。然而,m⁶A并非仅能促进转录本出核,其对mRNA出核转运的作用非常复杂,可能受到特定场景和特定因子的多重调控^[19,94]。除m⁶A以外,5-甲基胞苷(5-methylcytosine, m⁵C)也有类似的调控功能。m⁵C在3' UTR上的高密度分布可以特异性招募出核因子ALYREF,作为一种保守的机制促进mRNA出核^[95-98]。

综上所述,3' UTR延长介导的mRNA核滞留可视为滞留阻力与出核驱动力之间动态平衡的结果。3' UTR的长度及其携带的顺式滞留元件与因子构成了主导的滞留阻力,而同时其序列上的RNA修饰与出核蛋白互作提供了一定的出核驱动力^[99]。两种作用的竞争使带有长3' UTR的mRNA虽然具备出核能力,但整体仍倾向富集于核内(图2)。

4.3 3' UTR延长调控mRNA核滞留的生物学功能

3' UTR延长介导的mRNA核滞留也可作为细胞提供重要的转录本应急储备。研究表明,小鼠溶质

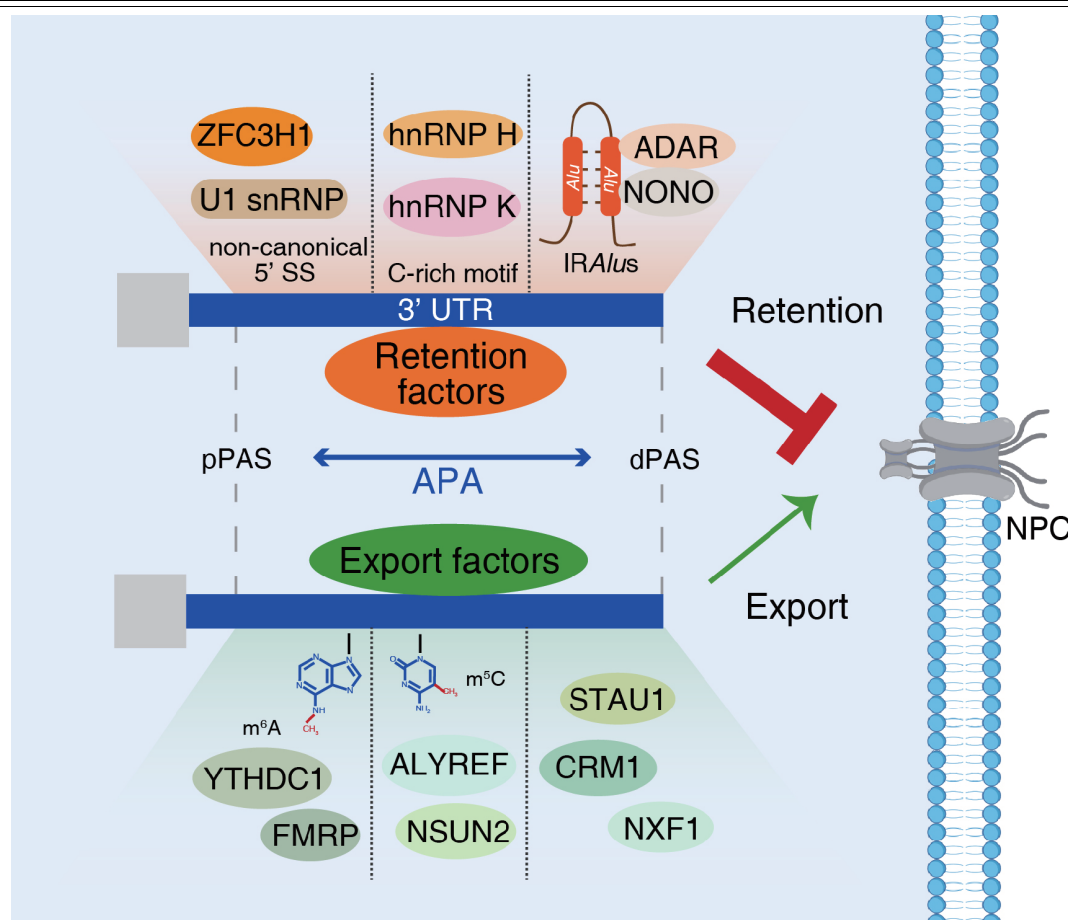


图2 3' UTR在mRNA核滞留中的调控作用

长3' UTR上不同的结构、序列与修饰能够招募并结合不同类型的蛋白因子,调控mRNA的核滞留。此外,发生在3' UTR的可变加尾事件则可以通过直接改变3' UTR长度,调整mRNA所携带的信息,影响转录本的核质分布。pPAS:近端加尾信号;dPAS:远端加尾信号。

Figure 2 Modulation of mRNA nuclear retention by long 3' UTR

Varied structures, sequences, and modifications on long 3' UTRs can recruit different protein factors to regulate mRNA nuclear retention. Additionally, alternative polyadenylation within the 3' UTR directly alters encoded information on the transcript by changing the length of 3' UTR, ultimately influencing mRNA's subcellular localization. pPAS: proximal polyadenylation signal; dPAS: distal polyadenylation signal.

载体家族7成员2(solute carrier family 7 member 2, *Slc7a2*)的长3' UTR携带IRAlus元件,因此被锚定在核旁斑中;当细胞受到应激刺激时,其mRNA的3' UTR会发生进一步切割,去除IRAlus元件,使mRNA脱离核滞留并释放进入细胞质,随后被翻译产生蛋白,从而满足应激状态下的基因表达需求^[100]。

3' UTR延长介导的核滞留还与细胞增殖与分化状态的改变相关。例如,在人胚胎干细胞中,由于核旁斑结构尚未形成,携带IRAlus的长3' UTR转录本不被扣留,能够顺利出核;当干细胞被诱导分化后,随着核旁斑逐渐组装形成,这些mRNA被有效地扣留在核内^[101]。这种依赖细胞状态的核滞留动态调控,可能有助于实现基因的时序性表达,并协同细

胞命运的切换。

此外,3' UTR延长介导的mRNA核滞留动态调控还可能与多种生理过程密切相关。以m⁶A修饰为例,由于其在3' UTR上高度富集且动态调控mRNA核质分布,因此m⁶A修饰很可能通过调控mRNA的核滞留参与多种生物学过程。例如,在ALKBH5缺失的小鼠中,mRNA的m⁶A修饰水平显著升高,导致大量原本滞留在核中的mRNA提前出核,最终引起精子发生异常、小鼠雄性不育^[93];相反,在肌肉组织中,m⁶A阅读蛋白YTHDC1的缺失会导致带有m⁶A修饰的mRNA大量滞留在核内,进而干扰骨骼肌干细胞的激活和增殖,造成肌肉损伤再生障碍^[91]。以上结果提示,3' UTR延长相关的核滞留调控在维持

mRNA核质分布平衡、促进生理功能正常发挥方面发挥重要作用。

在不同生理病理条件下,3' UTR的长度可以受到APA调控的直接影响,使得3' UTR延长介导的核滞留呈现出明显的基因特异性。在神经元发育过程中,ELAV家族蛋白偏好结合在尿嘧啶富集的近端PAS位点附近,通过物理占位直接干扰3'加工复合体对该位点的识别,进而驱动神经特异性转录本3' UTR的延长^[102]。这些长3' UTR mRNA滞留在核内,避免相关蛋白的过早合成,保障神经发育过程中基因表达的精确时序^[102]。而在肿瘤模型中,3'加工复合体成员Nudix水解酶21(nudix hydrolase 21, NUDT21)的水平明显降低,导致促癌基因蛋白酶体20S亚基 β 2(proteasome 20S subunit beta 2, PSMB2)和CXXC型锌指蛋白5(CXX5 finger protein 5, CXXC5)转录本的3' UTR变短,其mRNA出核被促进,蛋白水平显著上升,造成患者预后不良的后果^[103]。

综上所述,3' UTR延长介导的核滞留可能在多种生理病理过程中发挥调控作用。它不仅可以为细胞提供应激状态下的转录本储备,还参与细胞分化与命运转变等关键过程,保障了生物学过程的有序进行。当这一调控机制被破坏时,mRNA的核质分布可能发生失衡,造成基因表达紊乱,促进相关疾病的发生发展。

5 内含子保留与3' UTR延长协同调控mRNA核滞留

内含子保留和3' UTR延长除了能独立介导mRNA核滞留外,它们之间还存在协同调控的关系。剪接与3'端加工过程之间存在物理和功能上的紧密偶联^[104-106]。多个核心剪接因子与3'端加工复合体之间存在相互作用:例如,剪接因子U2 snRNP的相关亚基可与CPSF复合体成员互作^[107],从而将最末内含子的剪接状态与下游PAS的选择直接联系起来;同时,SR蛋白和hnRNP家族成员可作为分子桥梁,通过结合CstF或CFI复合体,在剪接体组装和加尾信号识别之间发挥调控作用^[108]。上述分子层面的交织,为剪接与3'端加工协同调控mRNA核滞留奠定了分子基础。

在这一分子偶联关系下,内含子保留与3' UTR延长往往同时发生,构成复合型的核滞留信号。靠近3'端的内含子剪接效率下降,可直接干扰3'端加

工复合体的组装,从而打破近端与远端加尾位点之间原有的平衡,使得远端加尾位点使用增加,产生更长的3' UTR^[109,110]。反之,远端加尾位点的选择推迟了转录终止,使得3'端区域剪接位点和相关调控元件的相互作用发生改变,影响末端内含子的识别与切除效率,从而增加转录本发生内含子保留的概率^[109,110]。这种剪接与加尾的双向反馈,使细胞能够协同利用剪接与加尾状态,实现对mRNA核滞留的动态调控^[2,4,111]。

从功能角度而言,内含子保留和3' UTR延长可以共同标记待加工状态的mRNA,通过双重机制确保它们的核滞留,为后续进一步加工提供了可能。在分子层面,内含子与长3' UTR可以增强mRNA与核内蛋白(如NONO、SON及SRRM2等无膜区室蛋白)的结合,从而将其扣留在染色质、核斑及核旁斑等亚核区域内^[112-114]。这些区域是mRNA出核前的质量监控场所,使核内剪接因子、3'端加工因子以及出核转运蛋白能够对滞留的mRNA进行进一步加工和出核分选^[112,113]。由此可见,内含子保留与3' UTR延长相互配合,共同强化了mRNA的核滞留与质量监控过程,确保了mRNA必须在适当的条件下完成加工,才能最终释放进入细胞质^[7,43,115]。

6 总结与展望

近年研究表明,mRNA核滞留不仅是转录加工质量控制的辅助机制,更是一种基因表达调控的重要手段。内含子保留与3' UTR延长作为引起mRNA核滞留的关键序列特征,通过影响RNA结合蛋白的招募及核内结构的相互作用,降低了mRNA的核输出效率,使转录本滞留在细胞核中,无法进入细胞质并发生翻译。这种核滞留有助于建立mRNA在功能性储备、质量监控与出核释放之间的动态平衡,为细胞提供了一种在核内对转录本进行“分选与决策”的重要调控层级。在这一机制下,mRNA的命运不再仅由其编码蛋白的功能决定,而是受到其内含子及3' UTR等非编码序列对转录本自身命运的显著影响。

然而,关于mRNA核滞留的分子机制与生物学意义,领域内仍存在诸多核心科学问题:首先,细胞如何整合剪接与3'端加尾等mRNA加工信号,并据此作出最终的转运决策;其次,核滞留究竟主要源于细胞对加工状态的主动识别,还是作为特定生理需

求进行的机动储备;此外,在某些情况下,核滞留是否还受到核孔复合体对特定形式RNA结构与构象的物理排斥。这些问题仍有待进一步研究解答。

针对上述核心问题,未来研究需要借助更高时空分辨率的技术手段,对mRNA核滞留的动态过程进行系统解析。单细胞转录组测序与长读长测序(long-read sequencing)技术将有助于系统描绘在不同生理状态下内含子保留和3' UTR延长的共同模式,量化不同序列特征对核滞留的贡献^[116-118]。同时,单分子RNA成像和活细胞追踪标记策略能够直观地揭示mRNA在细胞核内的停留动力学,从而验证特定的序列元件和互作蛋白如何影响出核与核滞留之间的平衡^[119,120]。此外,通过对亚细胞组分进行分离,并结合高分辨率邻近标记技术,有望进一步鉴定出连接mRNA加工、核滞留和出核转运过程的“分子桥梁”,阐明RNA结合蛋白如何识别不同的mRNA加工状态,进而参与出核或核滞留的决策^[121-123]。

深入理解mRNA核滞留这一调控层级,不仅有助于揭示真核生物基因表达的基本规律,也为解析因核质分布紊乱而引发的复杂疾病提供理论依据,从而进一步阐明真核细胞空间区室化在基因表达精细化调控中的重要意义。

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