What roles do different forms of RNA play in genome function?

非编码 RNA 研究概述

陈亮, 单革*

中国科学技术大学生命科学学院, 中国科学院天然免疫与慢性疾病重点实验室, 合肥 230027

* 联系人, E-mail: shange@ustc.edu.cn

2017-04-05 收稿, 2017-05-27 修回, 2017-05-27 接受, 2017-08-30 网络版发表

国家重点基础研究发展计划(2015CB943000)和国家自然科学基金(31471225, 31600657)资助

摘要 RNA是细胞以DNA为模板产生的转录产物, 根据中心法则, 早期一般将RNA整体地看作从DNA到功能蛋白质分子的中间信息专递分子. 这些分子也是较早为生物学家所认知的mRNA, rRNA, tRNA等. 其中mRNA直接作为翻译蛋白质的模板, 而rRNA及tRNA等的功能则直接保证蛋白质翻译的进行. 20世纪末及21世纪的研究逐渐让生物学家认识到细胞中还存在多种多样、对于中心法则遗传信息传递并非必需的非编码RNA分子. 认识RNA分子的种类、功能、机理, 及其与生理、遗传、进化等生命科学重要命题间的相互关系, 是当代生物学的重要内容. 本文对目前已知的非编码RNA种类、功能及机理, 以及在生理、遗传、进化、生态中的作用进行概述. 同时也简要介绍了非编码RNA相关的生物技术及生物医药应用. 非编码RNA研究已经取得了巨大的进展, 进一步的研究无疑将继续作为当代科学研究的重要领域存在, 从而回答各种各样RNA在基因组功能中的作用这一问题.

关键词 RNA, 非编码 RNA, 功能机理, 生理, 遗传, 进化

真核生物基因组具有广泛转录(pervasive transcription)的特性, 产生出大量的、各种各样的转录产物, 也就是RNA^[1,2]. 例如, 人类基因组至少78%的序列会产生相应的转录产物, 而这些转录产物中仅有不到2%的序列会作为模板将其序列所含信息“翻译”到产生的蛋白质氨基酸序列当中^[3,4].

一定意义上来说, 只有mRNA上的开放阅读框(open reading frame, ORF)序列才是“编码”序列, 而mRNA上的其他序列及大量的不能够作为翻译模板的非mRNA转录产物, 称为非编码RNA序列^[5-7]. 研究表明, mRNA上的非编码RNA序列可以调控mRNA的翻译、定位、稳定性等^[8-15]. 而研究大量的不作为翻译模板的所谓非编码RNA(noncoding RNA, ncRNA)序列的数量、种类、功能及其机理是近十几年来生物学的重要研究领域.

1 非编码RNA的种类、数量

在非编码RNA中、被认知和研究得较早的是核糖体RNA(ribosomal RNA, rRNA), 转运RNA(transfer RNA, tRNA)等所谓行使“管家”(house keeping)功能的分子. RNA对于几乎每个真核细胞的基本生命活动都必不可少. 例如, RNA剪切与修饰, mRNA翻译等. 细胞、特别是真核细胞当中, 除了多种“管家”(house keeping)非编码RNA以外, 还存在着大量的、各种各样的其他非编码RNA. 这些非编码RNA往往并非在各种真核细胞中均具有, 而是具有细胞或者时空特异性的表达. 这些非编码RNA按长度大于或小于200 nucleotide(nt)可以人为分为长链非编码RNA(long noncoding RNA, lncRNA)和小非编码RNA(有时也简称为小RNA, small noncoding RNA)^[16-18]. 进一步按照其序列特征、生成(biogenesis)、功能、功能

引用格式: 陈亮, 单革. 非编码 RNA 研究概述. 科学通报, 2017, 62: 3236-3244

Chen L, Shan G. A brief introduction of noncoding RNA research (in Chinese). Chin Sci Bull, 2017, 62: 3236-3244, doi: 10.1360/N972017-00384

机理等特性,无论是长链非编码RNA和小非编码RNA均可以分为多种类型。

最早作为一类小RNA被发现的是microRNA^[19~22];随后是广为人知的piRNA(piwi-interacting RNA)^[23,24]。而最早发现的长链非编码RNA(long non-coding RNA, lncRNA)为H19和XIST^[25~28]。随着物种进化程度的递增、小RNA(特别是microRNA)及长链非编码RNA的数量也逐步递增。人类具有数以千计的microRNA及数以万计的lncRNA。

近年来,被发现在真核多细胞生物中还存在一类大量的非编码RNA,即环形RNA(circular RNA, circRNA)^[29~31]。目前普遍认为真核细胞中绝大部分环形RNA是由向后剪切(back splicing)形成的。在人(*Homo sapiens*)、小鼠(*Mus musculus*)几乎各种细胞当中,环形RNA的种类可能均达到万种以上。

近10年来,随着二代测序(next generation sequencing, NGS)及生物信息学的发展,大量多种多样的非编码RNA被陆续发现。仅就本研究组而言,近几年已发现及命名了外显子-内含子环形RNA(exon-intron circular RNA, EIciRNA), 5S-OT(5S overlapped transcript) lncRNA, ASAT siRNA(α -satellite siRNA)等^[32~34]。需要特别指出的是,根据某一大类非编码RNA中的转录、加工、降解、序列、细胞内定位、功能及功能机理等特性,往往又可以分出不同的小类型(subclass)。例如,piRNA中有一类被称为pachytene piRNA,其即具有别于其他piRNA的一些特性。环形RNA中也有一类特殊的subclass,即EIciRNA。

从种类上,典型的哺乳动物细胞可能具有分别以万计的、序列不同的小RNA,长链非编码RNA以及环形RNA;而其中每种序列不同的非编码RNA的分子数量又可以是从小一到数以万计。完全可以预测未来还将发现大量的、新的非编码RNA。

2 非编码RNA的功能与功能机理

非编码RNA以非常多样的分子机制行使着各种各样的调控功能。在真核细胞中,非编码RNA大致可以分为在细胞核内或在细胞质中行使功能这两种类型。在细胞质中,研究得相对较为系统的是microRNA对mRNA的转录后调控。结构生物学已经揭示了microRNA通过结合AGO2对mRNA转录后调控的具体反应机理^[35,36]。一些长链非编码RNA也在细胞质中发挥作用,其中较多的例子是作为竞争性

内源RNA(competing endogenous RNA, ceRNA)来竞争性结合microRNA; ceRNA与microRNA的结合部分解除了相应microRNA对其靶mRNA的负调控作用^[37]。近年来,发现大量存在的环RNA也大量存在于细胞质中。其中有个别特例的环RNA以ceRNA的方式竞争性结合microRNA^[38,39]。当然,非编码RNA,无论小RNA还是长链非编码RNA,在细胞质中还有着多种其他调控方式。例如,1/2-sbsRNA及BACE1-AS调控mRNA的稳定性;lincRNA-p21及AS Uchl1调控mRNA翻译;NKILA调控IkB蛋白磷酸化等^[40~43]。在此不一一详述。

非编码RNA功能机理的特殊之处在于往往不能简单对某一类非编码RNA一概而论。仅就microRNA而言,已有报道一些microRNA并非通过结合mRNA 3'UTR来行使转录后的抑制功能。例如,有报道microRNA可以在线粒体中通过microRNA/AGO2复合物的方式起到促进线粒体基因表达的作用,但对于其详细机理并不非常清楚^[44,45]。

还有少数以前认定的所谓长链“非编码RNA”被发现实际上是编码小肽的“mRNA”^[46]。早期往往把最大可能ORF小于100个氨基酸残基、尚未有报道能编码多肽的RNA被认定为非编码RNA。目前被认定编码小肽的几种所谓非编码RNA均为在早期进行大规模鉴定时被归类为非编码RNA。目前就转录本已经可以对小至10个氨基酸残基的潜在开放阅读框进行物种间保守性分析,基本上可以很大程度上消除将编码小肽的转录本定义为非编码RNA的可能。目前看来,绝大部分在细胞质中现被鉴定为非编码RNA的转录本应该是起着非编码的功能。最近有研究发现,一些环形RNA在细胞质中也会作为翻译多肽的模板^[47~50],进一步的研究将弄清细胞质中有多大比例环形RNA可以作为翻译的模板,以及这些能作为翻译模板的环形RNA是以其翻译出的多肽还是依然以其作为RNA分子行使主要生物学功能。

转录、加工生成的RNA如果不被运出细胞核、基本上是起着非编码的功能。已知细胞核内非编码RNA可以通过与DNA、蛋白质以及RNA-RNA之间的相互作用来行使调控功能。基本上细胞核内的所有已知的主要调控均能看到非编码RNA的参与。例如,enhancer RNA(eRNA)可能参与了染色质的远程相互作用;hotair RNA参与了组蛋白修饰调控;Malat1及人5S-OT参与了mRNA剪切调控;diRNA参与了DNA

的损伤修复; piRNA参与了异染色质形成及mRNA降解等^[51~56]。有趣的是, 对于有些非编码RNA, 其RNA分子本身并无调控功能, 而是产生该转录本的转录过程或转录事件起着调控作用。无论是在细胞质, 还是在细胞核内, 很可能还有多种非编码RNA的调控方式尚待揭示。

3 非编码RNA的生理作用

大量证据显示, 非编码RNA在动物个体水平上起着重要的生理调控功能。其中研究得较早也较多的是, 雌性哺乳动物(或人类女性)中长链非编码RNA XIST在X染色体随机失活中的关键作用。表达XIST的X染色体在以此非编码RNA为主介导的一系列表观遗传调控事件后被转变为Barr body, 从而完成了哺乳动物中的X染色体剂量补偿^[57,58]。有意思的是, 果蝇(*Drosophila melanogaster*)的X染色体剂量补偿也与长链非编码RNA调控密切相关^[59]。雄性果蝇中两个长链非编码RNA, rox1和rox2作为形成MSL剂量补偿复合物(MSL dosage compensation complex)的支架(scaffold)来行使功能。NEAT1和MALAT1分别是哺乳动物细胞中与paraspeckle和nuclear speckle形成及功能相关的长链非编码RNA。但是令人意外的是, 迄今NEAT1敲除小鼠仅发现具有黄体及乳腺形成缺陷, 而MALAT1敲除小鼠尚未发现具有任何明显表型^[60~62]。一些长链非编码RNA敲除的人或小鼠ES细胞以及其他细胞系中也显示出一定的表型, 但是对于绝大部分长链非编码RNA, 目前还缺乏在各种模式动物、小鼠及人个体水平生理作用的研究。

对于小RNA中piRNA和microRNA的生理功能有较多的研究。piRNA通路失活在秀丽线虫(*Caenorhabditis elegans*)、果蝇、小鼠中均会导致配子发生上的缺陷^[63,64]。很多microRNA的生理作用已经得到认识。在秀丽线虫(线虫)、果蝇以及小鼠中, 多个microRNA的突变具有相应表型。例如, 最先被认识到的两个microRNA, *lin-4*及*let-7*的突变线虫即分别具有明显发育迟滞和寿命减短^[65~67]。果蝇的microRNA *bantam*突变具有神经发育障碍以及个体偏小等表型^[68]。miR-1突变小鼠存在心律不齐甚至猝死等表型^[69,70]。也发现很多microRNA突变并无明显表型; 有时需要把一个特定microRNA簇(microRNA cluster)或者microRNA家族(microRNA family)整体敲除才会表现出明显的表型。例如, 敲除miR-183/96/182后,

小鼠会出现记忆损伤^[71]; 这种结果表明, microRNA各家族成员具有功能上的冗余(redundancy)^[72]。近年来还发现, 一些非编码RNA(研究得较多的是microRNA)存在于外泌体(exosome)中, 可以起到细胞间调控作用^[73,74]。从这个意义上, 非编码RNA可以起到细胞间信号分子样的生理作用。当然目前对于大量microRNA以及其他各种非编码RNA的研究还停留在细胞水平上, 对于绝大部分非编码的生理功能还缺乏认知。

4 非编码RNA与生态、获得性遗传、进化

非编码RNA还参与了物种间以及生物与环境相互关系。例如, 人工在大肠杆菌(*Escherichia coli*)中表达的双链RNA可以进入取食细菌的秀丽线虫细胞内, 并被加工为siRNA而起到RNA干扰的作用(feeding RNAi)^[75~77]。本研究组发现, 大肠杆菌自身表达的非编码RNA可以进入秀丽线虫细胞内调控基因表达^[78]。一些植物中的microRNA, siRNA可以进入取食这些植物的动物, 如某些昆虫等体内, 并且起到调控基因表达的作用^[79~81]。甚至有研究提出, 植物中的microRNA可以通过消化道吸收进入人和小鼠等哺乳动物细胞, 并且调控基因表达^[82]。这方面的研究还有许多问题尚待解答, 甚至还具有争议。

一些遗传现象是以非基因序列依赖的方式进行的, 这种遗传现象即是所谓非孟德尔遗传, 也可以称为获得性遗传或者拉马克遗传。一系列的证据显示, 非编码RNA参与了特定类型的获得性遗传。例如, 饥饿处理的秀丽线虫会表现出长寿, 而其十多代内的后代, 尽管没有经受饥饿, 依然会表现出长寿。现有证据表明, 小非编码RNA参与了这种跨代遗传(transgenerational inheritance)^[83]。利用feeding RNAi干扰秀丽线虫特定基因表达也会产生跨代遗传现象。在亲代进行feeding RNAi, 而子代不进行feeding RNAi的情况下, 至少10代以内的子代依然表现出特定基因表达受到干扰。小非编码RNA也参与了feeding RNAi后的跨代遗传现象。最近的研究表明, 来源于tRNA的片段参与了小鼠中的一种跨代遗传现象。高脂饲养的雄性小鼠会具有一系列糖脂代谢异常, 而其在正常饲料喂养下发育及生长的子代也会表现出某些糖脂代谢异常。随后的研究表明, 高脂饲养的雄性小鼠精子中具有较高水平的tRNA片段, 而受精卵中注入tRNA片段会导致出生后的小鼠具有糖

脂代谢异常^[84]。目前,对于各种拉马克遗传现象的机理还知之甚少,而各种非编码RNA也许在其中具有多种多样的作用。

非编码RNA比蛋白质更具有物种特异性^[85]。极少数小非编码RNA,如let-7等数种microRNA在整个动物界均保守,大部分小非编码RNA在序列上并非保守。总体而言,长链非编码RNA序列上的保守性比小非编码RNA更弱。本研究组报道了迄今为止唯一发现的从裂殖酵母(*Saccharomyces*)到人均具有的长链非编码RNA 5S-OT,而且发现,5S-OT在高级灵长类及人类中进化出了新的序列及功能^[33]。无论是小非编码RNA还是长链非编码RNA均具有大量某一类动物甚至某一物种特有的成员。有科学家认为,编码蛋白质的基因可能对于生命活动的重大事件具有必需的重要性,所以倾向于非常保守;而非编码RNA主要起到调控作用,是生命进化以及物种间不同之处的主要推动因子之一^[86]。

5 非编码RNA相关生物技术、生物医学

非编码RNA已经在生物技术和生物医药领域得到使用。早在siRNA作为RNA干扰分子被发现后,大量的实验研究即开始使用siRNA(或者其他最终能在细胞内产生siRNA的方式)进行基因表达敲低实验。自21世纪初以来,将siRNA以及microRNA发展为可用于治疗人类疾病药物的努力持续不断。目前研究发现,很多非编码RNA在疾病模型中可以作为标记物,预示着其可以作为治疗靶点。例如,mir-21被发现在近乎所有的肿瘤中都高表达^[87];小RNA簇(microRNA cluster) miR-182-96-183也被证实参与到多个肿瘤发生进程^[88]。长非编码RNA *PCNA3*在前列腺癌中高表达,检测*PCNA3*的PROGENSA *PCNA3*分子检测试剂已被美国食品药品监督管理局(Food and Drug Administration, FDA)批准^[89,90]。

早在20世纪90年代初,RNA适配体(RNA aptamer)作为可以结合特定配体(一般为蛋白质、小分子甚至细胞)的人工小非编码RNA概念被提出并被筛选出来^[91-93]。在实验研究中,RNA适配体经常被用于结合特异蛋白质。本研究组即利用一种我们改进的筛选方法,得到了能特异识别p53点突变蛋白的RNA适配体,而且此适配体在人肿瘤细胞中能部分回复p53点突变蛋白的功能^[94]。RNA适配体也可发展为用于治疗人类疾病的药物。目前已经有一种FDA批准的

RNA适配体药物用于临床,并有多种RNA适配体药物已经进入临床实验^[95,96]。

最近数年来取得实质性进展的、以非编码RNA为基础的生物技术是CRISPR(Clustered regularly interspaced short palindromic repeats)^[97-100]。CRISPR是很多细菌具有的、将外源DNA以重复序列方式整合入自身基因组特定位点,并在随后可以外源DNA重复序列为模板转录出非编码RNA (sgRNA);进而细菌利用sgRNA可以指导切割,随后再侵入具有相同序列的外源DNA。CRISPR实际上是细菌对抗外源DNA的一种天然免疫方式。目前CRISPR已大量应用于实验研究当中,其各种衍生方法被用来敲除基因、敲入基因或DNA片段、上调或下调基因表达。利用CRISPR治疗人类遗传性疾病的尝试也正在开展^[101-103]。

除以上较为广泛及大量被应用的非编码RNA相关技术,很多其他非编码RNA也正在被研发或者已经被应用于疾病诊断及生物技术^[104-106];在此不一赘述。

6 研究展望

目前生物学界基本上认同各种各样的非编码RNA存在于生命之中,其中很多(如果不是全部的话)起着重要功能。例如,16S核糖体RNA, RNase P RNA等起着RNA酶作用的RNA,是地球生命开始之初的RNA世界(RNA world)即具有的非编码RNA^[107]。随着生命的进化,各种各样的非编码RNA作为重要的精细调控物种特异性调控分子出现。非编码RNA甚至可能作为“中性”(中性漂变学说(neutral theory of molecular evolution))分子,为进化提供可能,为重要非编码RNA功能分子及新编码基因起源提供材料^[108]。

已被发现的非编码RNA种类繁多,功能及机理呈多样性。毫无疑问,将来还会发现更多类型的非编码RNA及更广泛多样的非编码RNA的功能和作用机理。而对于非编码RNA在遗传、进化、生态中的作用的认识也将逐渐深入。随着基础研究的进行,非编码RNA相关的生物技术及生物医学应用也必将得到进一步发展。Science于2005年提出125个科学前沿问题时,认为是未来四分之一世纪需要重点研究的。完全有理由相信,到2030年时,人类对各种RNA分子的多样的功能会有更加深入的认知,并且已经可以较为广泛地利用RNA作为生物医学和生物技术手段。

参考文献

- 1 Crick F H. On protein synthesis. Symp Soc Exp Biol, 1958, 12: 138–163
- 2 Geiduschek E P, Haselkorn R. Messenger RNA. Annu Rev Biochem, 1969, 38: 647–676
- 3 International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature, 2004, 431: 931–945
- 4 Ponting C P, Oliver P L, Reik W. Evolution and functions of long noncoding RNAs. Cell, 2009, 136: 629–641
- 5 Quinn J J, Chang H Y. Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet, 2016, 17: 47–62
- 6 Cech T R, Steitz J A. The noncoding RNA revolution-trashing old rules to forge new ones. Cell, 2014, 157: 77–94
- 7 Guttman M, Rinn J L. Modular regulatory principles of large non-coding RNAs. Nature, 2012, 482: 339–346
- 8 Lee J T. Epigenetic regulation by long noncoding RNAs. Science, 2012, 338: 1435–1439
- 9 Castel S E, Martienssen R A. RNA interference in the nucleus: Roles for small RNAs in transcription, epigenetics and beyond. Nat Rev Genet, 2013, 14: 100–112
- 10 Ulitsky I, Bartel D P. lincRNAs: Genomics, evolution, and mechanisms. Cell, 2013, 154: 26–46
- 11 Hu S, Wu J, Chen L, et al. Signals from noncoding RNAs: Unconventional roles for conventional pol III transcripts. Int J Biochem Cell Biol, 2012, 44: 1847–1851
- 12 Liu J, Valencia-Sanchez M A, Hannon G J, et al. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol, 2005, 7: 719–723
- 13 Meng L F, Chen L, Li Z Y, et al. Environmental RNA interference in animals. Chin Sci Bull, 2013, 58: 4418–4425
- 14 Fu X. Non-coding RNA: A new frontier in regulatory biology. Natl Sci Rev, 2014, 1: 190–204
- 15 Belew A T, Meskauskas A, Musalgaonkar S, et al. Ribosomal frameshifting in the CCR5 mRNA is regulated by miRNAs and the NMD pathway. Nature, 2014, 512: 265–269
- 16 Ravasi T, Suzuki H, Pang K C. Experimental validation of the regulated expression of large numbers of non-coding RNAs from the mouse genome. Genome Res, 2006, 16: 11–19
- 17 Batistaand P J, Chang H Y. Longnoncoding RNAs: Cellular address codes in development and disease. Cell, 2013, 152: 1298–1307
- 18 Lee J T, Bartolomei M S. X-inactivation, imprinting, and long noncoding RNAs in health and disease. Cell, 2013, 152: 1308–1323
- 19 Lee R C, Feinbaum R L, Ambros V, et al. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell, 1993, 75: 843–854
- 20 Wightman B, Ha I, Ruvkun G. Posttranscriptional regulation of the heterochronic gene *lin-14* by *lin-4* mediates temporal pattern formation in *C. elegans*. Cell, 1993, 75: 855–862
- 21 Ambros V. The functions of animal microRNAs. Nature, 2004, 431: 350–355
- 22 Bartel D P. MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell, 2004, 116: 281–297
- 23 Seto A G, Kingston R E, Lau N C. The coming of age for PIWI proteins. Mol Cell, 2007, 26: 603–609
- 24 Siomi M C, Sato K, Pezic D, et al. PIWI-interacting small RNAs: The vanguard of genome defence. Nat Rev Mol Cell Biol, 2011, 12: 246–258
- 25 Pachnis V, Belayew A, Tilghman S M. Locus unlinked to a fetoprotein under the control of the murine *raf* and *Rif* genes. Proc Natl Acad Sci USA, 1984, 81: 5523–5527
- 26 Poirier F, Chan C T, Timmons P M, et al. The murine H19 gene is activated during embryonic stem cell differentiation *in vitro* and at the time of implantation in the developing embryo. Development, 1991, 113: 1105–1114
- 27 Davis R L, Weintraub H, Lassar A B. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell, 1987, 51: 987–1000
- 28 Wutz A. Gene silencing in X-chromosome inactivation: Advances in understanding facultative heterochromatin formation. Nat Rev Genet, 2011, 12: 542–553
- 29 Sanger H L, Klotz G, Riesner D, et al. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci USA, 1976, 73: 3852–3856
- 30 Gould A R. Studies on encapsidated viroid-like RNA. II. Purification and characterization of a viroid-like RNA associated with velvet tobacco mottle virus (VTMoV). Virology, 1981, 108: 123–133
- 31 Kos A, Dijkema R, Arnberg A C, et al. The hepatitis delta (delta) virus possesses a circular RNA. Nature, 1986, 323: 558–560
- 32 Li Z, Huang C, Bao C, et al. Exon-Intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol, 2015, 22: 256–264

- 33 Hu S, Wang X, Shan G. Insertion of an Alu element in an lncRNA leads to primate specific modulation of alternative splicing. *Nat Struct Mol Biol*, 2016, 23: 1011–1019
- 34 Huang C, Wang X, Liu X, et al. RNAi pathway participates in chromosome segregation in mammalian cells. *Cell Discov*, 2015, 1: 15029
- 35 Chendrimada T P, Gregory R I, Kumaraswamy E, et al. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*, 2005, 436: 740–744
- 36 Birchler J A, Kavi H H. Molecular biology. Slicing and dicing for small RNAs. *Science*, 2008, 320: 1023–1024
- 37 Kumar M S, Armenteros-Monterroso E, East P, et al. HMGA2 functions as a competing endogenous RNA to promote lung cancer progression. *Nature*, 2014, 505: 212–217
- 38 Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*, 2013, 495: 333–338
- 39 Hansen T B, Jensen T I, Clausen B H, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*, 2013, 495: 384–388
- 40 Kim Y K, Furic L, Parisien M, et al. Staufen1 regulates diverse classes of mammalian transcripts. *EMBO J*, 2007, 26: 2670–2681
- 41 Faghihi M A, Modarresi F, Khalil A M, et al. Expression of a noncoding RNA is elevated in Alzheimer's disease and drives rapid feed-forward regulation of beta-secretase. *Nat Med*, 2008, 14: 723–730
- 42 Huarte M, Guttman M, Feldser D, et al. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. *Cell*, 2010, 142: 409–419
- 43 Liu B, Sun L, Liu Q, et al. A cytoplasmic NFκB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. *Cancer Cell*, 2015, 27: 370–381
- 44 Kren B T, Wong P Y, Sarver A, et al. MicroRNAs identified in highly purified liver-derived mitochondria may play a role in apoptosis. *RNA Biol*, 2009, 6: 65–72
- 45 Zhang X, Zuo X, Yang B, et al. MicroRNA directly enhances mitochondrial translation during muscle differentiation. *Cell*, 2014, 158: 607–619
- 46 Anderson D M, Anderson K M, Chang C L, et al. A micropeptide encoded by a putative long noncoding RNA regulates muscle performance. *Cell*, 2015, 160: 595–606
- 47 Yang Y, Fan X, Mao M, et al. Extensive translation of circular RNAs driven by *N*⁶-methyladenosine. *Cell Res*, 2017, 27: 626–641
- 48 Pamudurti N R, Bartok O, Jens M, et al. Translation of circRNAs. *Mol Cell*, 2017, 66: 9–21
- 49 Legnini I, Di Timoteo G, Rossi F, et al. Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis. *Mol Cell*, 2017
- 50 Abe N, Matsumoto K, Nishihara M, et al. Rolling circle translation of circular RNA in living human cells. *Sci Rep*, 2015, 5: 16435
- 51 Kim T K, Hemberg M, Gray J M, et al. Widespread transcription at neuronal activity-regulated enhancers. *Nature*, 2010, 465: 182–187
- 52 Li W, Notani D, Ma Q, et al. Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature*, 2013, 498: 516–520
- 53 Rinn J L, Kertesz M, Wang J K, et al. Functional demarcation of active and silent chromatin domains in human *HOX* loci by noncoding RNAs. *Cell*, 2007, 129: 1311–1323
- 54 Tsai M C, Manor O, Wan Y, et al. Long noncoding RNA as modular scaffold of histone modification complexes. *Science*, 2010, 329: 689–693
- 55 Brown J A, Valenstein M L, Yario T A, et al. Formation of triple-helical structures by the 3'-end sequences of MALAT1 and MENbeta noncoding RNAs. *Proc Natl Acad Sci USA*, 2012, 109: 19202–19207
- 56 Doksani Y, de Lange T. The role of double-strand break repair pathways at functional and dysfunctional telomeres. *Cold Spring Harb Perspect Biol*, 2014, 6: 345–356
- 57 Brockdorff N, Turner B M. Dosage compensation in mammals. *Cold Spring Harb Perspect Biol*, 2015, 7: a019406
- 58 Barr M L, Bertram E G. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. *Nature*, 1949, 163: 676–677
- 59 Borsani G, Tonlorenzi R, Simmler M C, et al. Characterization of a murine gene expressed from the inactive X chromosome. *Nature*, 1991, 351: 325–329
- 60 Nakagawa S, Ip J Y, Shioi G, et al. Malat1 is not an essential component of nuclear speckles in mice. *RNA*, 2012, 18: 1487–1499
- 61 Zhang B, Arun G, Mao Y S, et al. The lncRNA Malat1 is dispensable for mouse development but its transcription plays a *cis*-regulatory role in the adult. *Cell Rep*, 2012, 2: 111–123
- 62 Clemson C M, Hutchinson J N, Sara S A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. *Mol Cell*, 2009, 33: 717–726
- 63 Muller F L, Colla S, Aquilanti E, et al. Passenger deletions generate therapeutic vulnerabilities in cancer. *Nature*, 2012, 488: 337–342

- 64 Casey S C, Tong L, Li Y, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science*, 2016, 352: 227–231
- 65 Chalfie M, Horvitz H R, Sulston J E. Mutations that lead to reiterations in the cell lineages of *C. elegans*. *Cell*, 1981, 24: 59–69
- 66 Ambros V. A hierarchy of regulatory genes controls a larva-to-adult developmental switch in *C. elegans*. *Cell*, 1989, 57: 49–57
- 67 Meng L, Chen L, Li Z, et al. Roles of microRNAs in the *Caenorhabditis elegans* nervous system. *J Genet Genom*, 2013, 40: 445–452
- 68 Hipfner D R, Weigmann K, Cohen S M. The bantam gene regulates *Drosophila* growth. *Genetics*, 2002, 161: 1527–1537
- 69 Yang B, Lin H, Xiao J, et al. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med*, 2007, 13: 486–491
- 70 Zhao Y, Ransom J F, Li A, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell*, 2007, 129: 303–317
- 71 Woldemichael B T, Jawaid A, Kremer E A, et al. The microRNA cluster miR-183/96/182 contributes to long-term memory in a protein phosphatase 1-dependent manner. *Nat Commun*, 2016, 7: 12594
- 72 Song R, Walentek P, Sponer N, et al. miR-34/449 miRNAs are required for motile ciliogenesis by repressing cp110. *Nature*, 2014, 510: 115–120
- 73 Zhang L, Zhang S, Yao J, et al. Microenvironment-induced PTEN loss by exosomal microRNA primes brain metastasis outgrowth. *Nature*, 2015, 527: 100–104
- 74 Thomou T, Mori M A, Dreyfuss J M, et al. A dipose-derived circulating miRNAs regulate gene expression in other tissues. *Nature*, 2017, 542: 450–455
- 75 Fire A, Xu S, Montgomery M K, et al. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature*, 1998, 391: 806–811
- 76 Tabara H, Grishok A, Mello C C. RNAi in *C. elegans*: Soaking in the genome sequence. *Science*, 1998, 282: 430–431
- 77 Timmons L, Fire A. Specific interference by ingested dsRNA. *Nature*, 1998, 395: 854
- 78 Liu H, Wang X, Wang H D, et al. *Escherichia coli* noncoding RNAs can affect gene expression and physiology of *Caenorhabditis elegans*. *Nat Commun*, 2012, 25: 1073
- 79 Bellafiore S, Briggs S P. Nematode effectors and plant responses to infection. *Curr Opin Plant Biol*, 2010, 13: 442–448
- 80 Maule A G, McVeigh P, Dalzell J J, et al. An eye on RNAi in nematode parasites. *Trends Parasitol*, 2011, 27: 505–513
- 81 Lilley C J, Davies L J, Urwin P E. RNA interference in plant parasitic nematodes: A summary of the current status. *Parasitology*, 2012, 139: 630–640
- 82 Zhang L, Hou D, Chen X, et al. Exogenous plant mir168a specifically targets mammalian LDLRAP1: Evidence of cross-kingdom regulation by microRNA. *Cell Res*, 2012, 22: 107–126
- 83 Hourri-Ze'evi L, Korem Y, Sheftel H, et al. A Tunable mechanism determines the duration of the transgenerational small RNA inheritance in *C. elegans*. *Cell*, 2016, 165: 88–99
- 84 Chen Q, Yan M, Cao Z, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. *Science*, 2016, 351: 397–400
- 85 Pasquinelli A E, Reinhart B J, Slack F, et al. Conservation of the sequence and temporal expression of *let-7* heterochronic regulatory RNA. *Nature*, 2000, 408: 86–89
- 86 Haudry A, Platts A E, Vello E, et al. An atlas of over 90000 conserved noncoding sequences provides insight into crucifer regulatory regions. *Nat Genet*, 2013, 45: 891–898
- 87 Kumarswamy R, Volkmann I, Thum T. Regulation and function of miRNA-21 in health and disease. *RNA Biol*, 2011, 8: 706–713
- 88 Mihelich B L, Khramtsova E A, Arva N, et al. MiR-183-96-182 cluster is overexpressed in prostate tissue and regulates zinc homeostasis in prostate cells. *J Biol Chem*, 2011, 286: 44503–44511
- 89 Tinzi M, Marberger M, Horvath S, et al. DD3PCA3 RNA analysis in urine—A new perspective for detecting prostate cancer. *Eur Urol*, 2004, 46: 182–187
- 90 De Kok J B, Verhaegh G W, Roelofs R W, et al. DD3PCA3, a very sensitive and specific marker to detect prostate tumors. *Cancer Research*, 2002, 62: 2695–2698
- 91 Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*, 1990, 249: 505–510
- 92 Ellington A D, Szostak J W. *In vitro* selection of RNA molecules that bind specific ligands. *Nature*, 1990, 346: 818–822
- 93 Bock L C, Griffin L C, Latham J A, et al. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature*, 1992, 355: 564–566
- 94 Chen L, Rashid F, Shah A, et al. The isolation of an RNA aptamer targeting to p53 protein with single amino acid mutation. *Proc Natl Acad Sci USA*, 2015, 112: 10002–10007

-
- 95 Gragoudas E S, Adamis A P, Cunningham E T, et al. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med*, 2004, 351: 2805–2816
- 96 Ng E W, Shima D T, Calias P, et al. Pegaptanib, a targeted anti-VEGF aptamer for ocular vascular disease. *Nat Rev Drug Discov*, 2006, 5: 123–132
- 97 Horvath P, Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. *Science*, 2010, 327: 167–170
- 98 Marraffini L A, Sontheimer E J. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. *Nat Rev Genet*, 2010, 11: 181–190
- 99 Redman M, King A, Watson C, et al. What is CRISPR/Cas9? *Archives of disease in childhood. Educ Prac Ed*, 2016, 101: 213–215
- 100 Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, 2007, 315: 1709–1712
- 101 Modell J W, Jiang W, Marraffini L A. CRISPR-Cas systems exploit viral DNA injection to establish and maintain adaptive immunity. *Nature*, 2017, 544: 101–104
- 102 Shimokawa M, Ohta Y, Nishikori S, et al. Visualization and targeting of LGR5⁺ human colon cancer stem cells. *Nature*, 2017, 545: 187–192
- 103 Cloney R. Genetic screens: CRISPR-based mapping of genetic interactions. *Nat Rev Genet*, 2017, 18: 272–273
- 104 Shalem O, Sanjana N E, Hartenian E, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*, 2014, 343: 84–87
- 105 van Diemen F R, Kruse E M, Hooykaas M J, et al. CRISPR/Cas9-mediated genome editing of herpesviruses limits productive and latent infections. *PLoS Pathog*, 2016, 12: e1005701
- 106 Liu Y, Zhan Y, Chen Z, et al. Directing cellular information flow via CRISPR signal conductors. *Nat Methods*, 2016, 13: 938–944
- 107 Gilbert W. The RNA world. *Nature*, 1986, 319: 618
- 108 Kimura M. Rare variant alleles in the light of the neutral theory. *Mol Biol and Evolution*, 1983, 1: 84–93

单 革

博士，中国科学技术大学生命科学学院教授。1995 年于兰州大学获得细胞生物学学士学位，1998 年于中国科学院上海细胞生物研究所获得硕士学位，2005 年于美国佐治亚州立大学获得博士学位。先后在美国爱默生(Emory)大学和耶鲁大学进行博士后研究(师从 RNA 酶共同发现人 Sidney Altman 教授)。2010 年进入中国科学技术大学工作。长期从事基因表达调控和非编码 RNA 功能及功能机理方面的研究。发现了新的非编码 RNA 类型(如 EIciRNA, 5S-OT, ASAT siRNA 等)，研究了新的非编码 RNA 功能，揭示了新的非编码 RNA 作用机理，探索了新的非编码 RNA 研究方法。实验室成立以来在 *Nat Struct Mol Biol* (2015; 2016), *Nat Commun* (2012)和 *Proc Natl Acad Sci USA* (2015)发表多篇非编码 RNA 领域的研究论文。

Summary for “非编码 RNA 研究概述”

A brief introduction of noncoding RNA research

CHEN Liang & SHAN Ge*

CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Life Sciences, University of Science and Technology of China, Hefei 230027, China

* Corresponding author, E-mail: shange@ustc.edu.cn

RNAs are products of transcription with DNA as their template. In early days of molecular biology and as suggested in the central dogma, RNAs had been viewed as information carrier to transfer genetic information from DNA to functional proteins. This is true for those RNAs discovered earlier by biologists such as mRNAs, rRNAs, tRNAs, snRNAs, and etc. mRNAs are template of translation to give rise to proteins, whereas snRNAs, rRNAs, tRNAs, and some other noncoding RNAs (ncRNAs) are either directly responsible for the correct produce of mRNAs or essential molecules in the process of protein translation. Starting around the last decade of the 21st century and continuing in this century, biologists have increasingly realized that a great number of diverse ncRNAs are present in cells, and they are versatile molecules but not essential for the information flow suggested by the central dogma. RNAs were presumably precursor molecules in first life forms on earth, as proposed in the RNA world hypothesis. Over the period of evolutionary time, the genetic information was stored in more stable DNA, while most catalytic and structural functions were handed over to more versatile proteins. Some RNAs still function as essential enzymes in current life forms, and a lot RNAs function as intermediates between genes and proteins. On the other hand, pervasive transcription of eukaryotic genome generates abundant ncRNAs. Accuminating findings demonstrate that a lot of ncRNAs are critical regulators in a plethora of life events, which also suggests that ncRNAs may be one of major driving forces in the evolution of life. The first wave in ncRNA studies is on small noncoding RNAs, e.g. microRNAs and piRNAs; then the field of long noncoding RNAs emerges. It has become one of the focuses of modern biology to know the inventory, functions, functional mechanisms, physiological significances, ecological roles, relevance to inheritance, and involvement in evolution of ncRNAs. Here we briefly introduce our current understandings about categories of ncRNAs. An enormous number of small, long, and more recently circular, ncRNAs are identified in eukaryotic cells. They function in the cytoplasm, in the nucleus, intercellularly, or even interspecies, with a great variety of mechanisms. We have a concise summary about their roles in physiology, inheritance, evolution, and ecology. NcRNAs generally function through interacting with proteins, DNA, or other RNAs (coding or noncoding). We also have a short discussion about biotechnologies and biomedical applications derived from ncRNAs. It is speculated that more ncRNA categories (or subtypes, or novel members of known category) will be identified in the future. New roles and functional mechanisms will also be uncovered. Great progresses have been made in the field of ncRNAs, and to decipher ncRNAs will undoubtedly remain to be a significant area of research for the coming decades, which would eventually answer the question of “what roles do different forms of RNA play in genome function”.

RNA, noncoding RNA, functional mechanism, physiology, inheritance, evolution

doi: 10.1360/N972017-00384