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·特邀综述·

染色质可及性与植物基因表达调控

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摘要 真核生物基因组上的核小体呈现不均匀分布, 转录活跃区域的染色质结构相对松散且易被调节蛋白结合, 这些区域的可接近程度称为染色质可及性。随着测序技术的发展, DNase-seq、ATAC-seq、MNase-seq和NOMe-seq等组学技术的应用, 全基因组范围内染色质可及性检测变得简便且高效。该文主要介绍了真核生物染色质可及性的4种基本检测方法的技术原理, 总结了核小体定位、组蛋白修饰以及转录因子结合与染色质可及性的关系, 并综述了染色质可及性参与植物生长发育和环境响应研究进展, 以期为植物领域全基因组水平染色质可及性研究、顺式调控元件挖掘及发育和环境响应过程中基因表达调控网络的解析提供借鉴。

关键词 染色质可及性, 组蛋白修饰, DNase-seq, ATAC-seq, 调控元件

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真核生物基因组DNA与组蛋白结合缠绕形成核小体, 构成染色质复杂三维结构的基本单元。单个核小体为长约147 bp的DNA以左螺旋方式缠绕在组蛋白八聚体上, 而核小体之间的DNA称作连接DNA (linker DNA) (Luger et al., 1997)。整个基因组上核小体的分布并不均匀, 转录不活跃的异染色质区核小体的排列相对致密; 而在一些转录活跃区(如启动子、增强子和正在转录的基因区)核小体的排列相对松散。该区域的核小体间DNA往往被转录因子和RNA聚合酶等调节蛋白结合, 从而形成更复杂的染色质高级结构(Lee et al., 2004; Thurman et al., 2012)。相对松散且易被调节蛋白结合的区域为开放染色质区, 其可被调节蛋白结合的程度称为染色质可及性, 是核小体定位、转录因子结合和染色质重塑因子等综合作用的结果(Klemm et al., 2019)。细胞中染色质可及性在生长发育和外界刺激过程中呈现动态变化。越来越多的证据表明, 染色质可及性与基因的表达调控、生物体的发育分化和环境响应有密切联系(Thurman et al., 2012; Sullivan et al., 2014; Qiu et al., 2016; Sijacic et al., 2018; Zeng et al., 2019)。

随着高通量测序技术的发展, 染色质可及性检测技术也有了长足进步, 人们有望深入理解染色质结构及其参与基因表达的调控模式。本文主要介绍了真核生物染色质可及性的检测方法, 总结了核小体定位、组蛋白修饰和转录因子结合与染色质可及性的关系, 并综述了染色质可及性参与植物生长发育和环境响应的研究进展, 以期为植物领域全基因组水平染色质可及性研究、顺式调控元件挖掘, 及发育与环境响应过程中基因表达调控网络的解析提供借鉴。

1 染色质可及性检测方法

1.1 检测技术及其发展历程

染色质可及性高的区域DNA呈开放状态, 容易成为核酸酶的靶标位点。染色质可及性检测主要通过染色质DNA对各类核酸酶降解作用的敏感程度来衡量, 这些核酸酶包括脱氧核糖核酸酶I (deoxyribonuclease, DNase I)、微球菌核酸酶(micrococcal nuclease, MNase)和转座酶(transposase)等。早在20世纪70年代, 研究人员就开始用DNase I和MNase对染色质进

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行消化进而研究其结构, 结果发现经过高浓度核酸酶消化后的染色质出现100–200 bp的周期性片段(Noll, 1974; Lohr et al., 1977)。染色质不同区域对DNase I的敏感性不同, 当一个基因处于转录活性状态时, 其对DNase I降解作用的敏感性比无转录活性区域高出100倍以上, 称为DNase I超敏感位点(DNase I hypersensitive site, DH site), 即开放染色质区(Wu, 1980; Keene et al., 1981; McGhee et al., 1981)。最初, 研究人员对酶切产生的染色质DNA片段加以纯化, 采用Southern blot通过特定序列的探针对切割位点进行分析, 从而实现对特定位点的染色质可及性检测(Nedospasov and Georgiev, 1980; Wu, 1980; Conconi and Ryan, 1993)。之后, 伴随着PCR技术的引入, 一系列qPCR和克隆文库构建的定量检测方法相继被开发出来(Mueller and Wold, 1989; Rao et al., 2001)。2004年, Stamatoyannopoulos和Collins两个研究团队分别采用全基因组DH位点平行克隆方法, 将开放染色质研究推进到全基因组水平(Crawford et al., 2004; Sabo et al., 2004)。Boyle等(2008)开创性地将DNase I部分酶解后的染色质DNA直接用于二代测序文库的构建, 并进行高通量测序分析。至此, 染色质可及性检测正式进入基于二代测序的高通量时代, 分析效率和规模均得到极大的提升。高通量测序技术的发展, 推动了多种可用于染色质可及性检测方法的出现, 如DNase-seq、ATAC-seq、MNase-seq和NOMe-seq (图1)。

1.2 DNase-seq

DNase-seq (deoxyribonuclease I hypersensitive site sequencing)是利用DNase I对细胞核染色质进行部分酶解, 并对回收后的DNA进行二代测序, 从而在全基因组水平分析染色质可及性的一种方法(图1A)。该方法的建立可追溯到2006年, Crawford等(2006)和Sabo等(2006)同时报道了利用基因芯片技术实现人类全基因组2%区域的开放染色质检测。随后, 伴随二代测序技术的发展, 这两种染色质可及性检测策略被应用到高通量领域, 从而真正实现了全基因组水平的染色质可及性检测。这两种策略可简单概括为末端捕获法(end-capture) (Boyle et al., 2008)和双酶切法(double-hit) (Hesselberth et al., 2009)。末端捕获法最早由Boyle等(2008)提出, 该方法通过使用适量DNase I酶处理细胞核染色质, 使其在开放染色质区形成切割位点, 然后借助限制性内切酶Mme I的特异性切割作用, 捕获切口末端长度为20 bp的序列用于染色质可及性分析。双酶切法则由Hesselberth等(2009)提出并首次应用于酵母(*Saccharomyces cerevisiae*)调控蛋白结合印记作图(Hesselberth et al., 2009)。该方法通过将DNase I的用量控制在一定范围内, 使其在调节蛋白两侧形成双酶切, 从而将DNA切割成小片段释放出来, 进一步对这些小片段进行测序分析来评估该区域染色质可及性。相较而言, 末端捕获法可获得的开放染色质位点更多, 双酶切法则提供了一种相对简便的操作流程和更高的信噪比(Klemm et al., 2019)。同时, 双酶切法用于测序的DNA片段长度(50–150 bp)远大于末端捕获法(20 bp), 可有效克服复杂基因组比对率低的问题。尽管如此, 二者在大范围尺度上仍有较好的一致性。

1.3 ATAC-seq

ATAC-seq (assay for transposase accessible chromatin with high-throughput sequencing)方法利用一种超高活性的转座酶Tn5将开放染色质区域的DNA片段化, 并加上接头(图1B)。该方法由Buenrostro等(2013)在人类细胞上建立, 原理与DNase-seq的双酶切法类似, 均为将开放染色质区DNA切割成小片段用于二代测序分析。采用ATAC-seq方法分析染色质可及性结果与DNase-seq末端捕获法和双酶切法均有很高的相似性(r>0.75和r>0.8) (Buenrostro et al., 2013; Corces et al., 2017)。但值得注意的是, 在高分辨率的转录因子结合印记(TF footprint)分析过程中, ATAC-seq和DNase-seq均表现出一定的偏好性, 且偏好序列因2种酶的识别特性不同而异(He et al., 2014; Meyer and Liu, 2014; Schep et al., 2015)。ATAC-seq相较于DNase-seq方法的优势在于, Tn5转座酶在介导染色质片段化的同时也将测序接头连入, 这一特性使其文库构建变得十分简便和高效。通常, 仅需2小时便可完成文库构建的整个流程, 其样品起始使用量也可低至500个细胞(Buenrostro et al., 2013; Corces et al., 2017)。鉴于上述优势, ATAC-seq方法成为染色质可及性检测领域的热门技术之一。

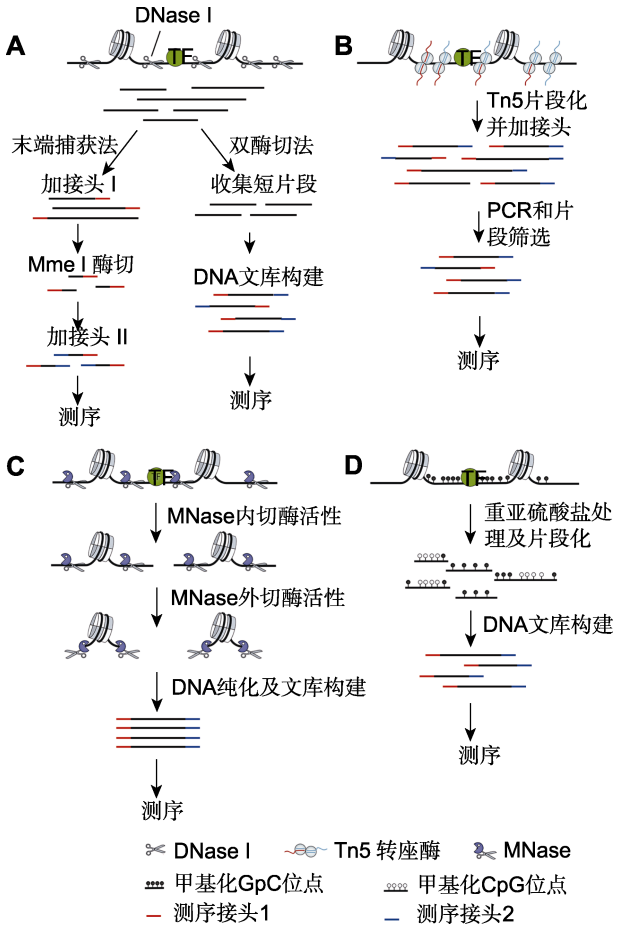

图1 常见染色质可及性检测方法

(A) DNase-seq方法是利用核酸内切酶DNase I消化开放染色质区DNA并进行建库的方法。其中，末端捕获法通过提取单酶切后的染色质DNA大片段，经加测序接头1、Mme I酶切和加测序接头2等步骤，完成含有插入片段20 bp的测序文库构建；双酶切法则通过提取双酶切后的染色质小片段，经加测序接头1和2完成测序文库的构建。**(B)** ATAC-seq方法利用Tn5超敏感转座酶的高效转座活性在完成开放染色质区DNA打断的同时加入测序接头，实现高效文库构建。**(C)** MNase-seq方法利用MNase的内切酶和外切酶活性将开放染色质区DNA切开，同时消化linker DNA，构建核小体DNA文库，可实现核小体占位及染色质可及性的同时检测。**(D)** NOMe-seq方法利用GpC甲基转移酶对开放染色质区GpC二核苷酸进行甲基化修饰。人类细胞中仅存在CPG^m位点而无内源的GpC^m位点，经亚硫酸氢盐处理后进行全基因组测序，即可同时检测开放染色质区及全基因组的DNA甲基化位点。

Figure 1 Principal methods for measuring chromatin accessibility

(A) DNase-seq uses the endonuclease DNase I to cleave DNA within accessible chromatin and constructs the DNase-seq library. In the end-capture method, the large DNA fragments from open chromatin regions released by single cut are collected, and then processed by several steps including adding adapter 1, cutting 20 bp of the DNA using a type II restriction enzyme Mme I, and adding adapter 2 to complete the sequencing library. In the double-hit method, the small DNA fragments from open chromatin regions released by double cut are collected, then processed by steps including adding adapter 1 and adapter 2 to complete the sequencing library; **(B)** ATAC-seq uses a hyperactive transposase (Tn5) to simultaneously cleave and ligate adaptors to accessible DNA. The efficiency of library construction is thus increased; **(C)** MNase-seq uses the endonuclease/exonuclease activity of MNase to both cleave and eliminate accessible DNA and linker DNA. Therefore, the DNA library from nucleosomal DNA can both detect the nucleosome occupancy and open chromatin; **(D)** NOMe-seq uses a GpC methyltransferase to methylate GpC dinucleotide in accessible DNA. There is only CPG^m sites but not GpC^m sites in human cells. The DNA sequencing following bisulfite conversion of nonmethylated cytosine to uracil nucleotides can simultaneously provide the genome-wide measure of accessibility and DNA methylation sites.

1.4 MNase-seq

MNase-seq (micrococcal nuclease sequencing)是利用微球菌核酸酶消化核小体间连接DNA，从而对核小体定位情况进行分析的技术(图1C)。鉴于核小体占位在染色质可及性研究过程中发挥十分重要的作用，Mieczkowski等(2016)和Mueller等(2017)利用MNase-seq方法研究了染色质可及性。MNase可作为核酸内切酶切割核小体间的linker DNA，也可作为核酸外切酶消化未被蛋白质保护的DNA部分。但整体而言，MNase对核小体DNA的切割效率低于核小体间DNA (Chung et al., 2010; Lorzadeh et al., 2016)，这也是该酶被广泛用于核小体定位研究的原因。但Mieczkowski等(2016)研究发现一些核小体也表现出对MNase剂量敏感，利用MNase的这一特性他们开发了MACC (MNase accessibility)方法(Mieczkowski et al., 2016)。该方法可对整个基因组上的核小体占位及染色质可及性同时进行检测，用于探究两者间的互作关系。MACC检测信号与DNase-seq结果大体一致，在转录活性位点(如转录起始位点(transcription start site, TSS)、启动子区和增强子区)均检测到开放染色质信号(Mieczkowski et al., 2016; Mueller et al., 2017)。不同的是，在转录终止区(transcription termination site, TTS)和基因区却很少检测到开放染色质信号，产生这一差异的原因可能与用于测序的DNA

片段回收筛选过程或DNase I和MNase两种核酸酶分子量的大小有关。此外, Zhao等(2020)研究表明, 利用MNase可检测到DNase I或Tn5检测不到的开放染色质区域。

1.5 NOMe-seq

NOMe-seq (nucleosome occupancy and methylome sequencing)借助GpC甲基转移酶(GpC methyltransferase, MTase) M.CviPI对开放染色质区GpC二核苷酸进行甲基化修饰的作用来检测染色质可及性(图1D)。该方法由Kelly等(2012)在人类细胞上建立, 原理与DNase-seq、ATAC-seq和MNase-seq方法不同。NOMe-seq不对染色质DNA进行切割, 而是利用MTase对开放染色质区GpC二核苷酸的化学修饰能力来分析染色质可及性。由于人类细胞中不存在内源的GpC^m位点, 故经过亚硫酸氢盐处理和全基因组测序后, 可同时获得细胞内GpC和CpG两种二核苷酸的相关信息(分别来自MTase对开放染色质区域GpC的修饰及全基因组DNA的甲基化位点)。因此, 该方法可以同时获得染色质可及性和全基因组甲基化信息(Kelly et al., 2012)。值得注意的是, 该方法不需要对染色质DNA进行切割, 也不需要对DNA片段进行富集, 需要大量测序数据以获得足够的测序深度和基因组覆盖率, 才能获得整个基因组水平的染色质可及性数据。当然, 正是由于其不切割DNA的特性, 该方法有效避免了切割和富集带来的偏好性问题。

2 影响染色质可及性的因素

真核生物基因组DNA被组蛋白或其它DNA结合因子(包括转录因子、染色质重塑因子和其它结构性蛋白等)覆盖。染色质可及性是这些结合蛋白动态竞争性结合的结果。下面对决定染色质可及性的关键因素(包括核小体定位、组蛋白修饰和转录因子结合等)与染色质可及性的相互关系进行介绍。

2.1 核小体定位与染色质可及性

核小体覆盖真核生物基因组的大部分区域, 以重复、有规律的方式排列在染色质上。但核小体在基因组上的组织方式并非一成不变, 而是动态变化的。这种动态变化表现为核小体在DNA上的动态滑动, 自发地发生部分或全部解聚(Lai and Pugh, 2017)。核小体在不同染色质区域的解聚速率不同, 表现为活跃的启动子和增强子区核小体解聚速率明显高于不活跃的异染色质区。核小体的定位和解聚与许多调节因素有关, 包括DNA序列特异性、组蛋白变体的参与、染色质重塑复合物、分子伴侣和转录因子等(Rando and Ahmad, 2007; Klemm et al., 2019)。研究发现, 在酿酒酵母启动子区域普遍存在1个核小体缺失位点(nucleosome-free region, NFR), 核小体的缺失增强了该位点的染色质可及性, 使其具有更强的调控潜力, 有利于调控蛋白的结合(Almer and Hörz, 1986; Yuan et al., 2005)。NFR上下游边界各存在1个固定的核小体结构, 命名为–1/+1核小体。+1核小体定位于转录起始位点下游的固定距离, 且该距离在不同物种中的长度不同(Yuan et al., 2005; Mavrich et al., 2008a, 2008b; Schones et al., 2008)。将启动子区核小体的排列情况与RNA聚合酶II的ChIP-seq结果进行联合分析, 发现启动子区域RNA聚合酶II占据水平越高, +1核小体及其下游的核小体定位现象越明显(Mavrich et al., 2008a), 说明+1核小体在RNA聚合酶II参与的转录中起重要作用。而–1核小体位置上往往结合有SWI/SNF (switch/sucrose nonfermenting)复合物(Dutta et al., 2014)。SWI/SNF是一类研究较为全面的染色质重塑复合物, 包含一类依赖ATP的蛋白酶, 可利用ATP水解的能量移除、移动或引入组蛋白, 从而改变核小体的定位与构象(Ribeiro-Silva et al., 2019), 进而将调控元件DNA序列暴露出来, 以利于转录因子与DNA的结合。另一类染色质重塑复合物SWR1 (Swi2/Snf2-related1)可介导组蛋白变体H2A.Z在染色质上沉积, 从而替换常规的H2A蛋白, 在酵母、果蝇(*Drosophila melanogaster*)、人(*Homo sapiens*)和拟南芥(*Arabidopsis thaliana*)等真核生物中均发现H2A.Z于+1核小体位置大量沉积(Clapier et al., 2017; Kumar, 2018)。此外, Luo等(2020)和Murphy等(2020)发现H2A.Z蛋白在基因组特定区域的组装与移除也可对染色质结构产生重要影响, 从而参与多种生物学过程中的基因表达调控。

2.2 组蛋白修饰与染色质可及性

核小体组蛋白修饰也称作组蛋白密码(histone code), 可被细胞内特定的蛋白质识别, 从而改变和影响染色

质的物理结构(Rothbart and Strahl, 2014)。因此，核小体组蛋白的修饰状态往往与染色质功能的激活/抑制密切相关。常见的组蛋白修饰状态包括赖氨酸乙酰化、甲基化和泛素化，精氨酸的甲基化以及丝氨酸的磷酸化(Felsenfeld and Groudine, 2003)。从分子极性角度分析，乙酰化修饰可在一定程度上削弱组蛋白与DNA分子间的相互作用(Vogelauer et al., 2000; Bernstein et al., 2002)，从而加快核小体组蛋白的解聚速率，提高染色质可及性，使转录因子更容易与调控元件结合。研究发现，乙酰化修饰通常与转录活性高的启动子有较高的关联度。例如，启动子H3K9ac修饰水平与促进人类干细胞分化相关基因的表达水平呈正相关(Du et al., 2017)。与乙酰化修饰不同，组蛋白甲基化修饰使核小体与组蛋白结合更加稳定，且甲基化修饰有一甲基化、二甲基化和三甲基化3种形式(Rothbart and Strahl, 2014)。不同的甲基化修饰作用不同，有些甲基化修饰可促进转录。例如，H3K4me3在人类细胞中主要分布于活跃转录基因的启动子处(Vallianatos et al., 2020)，这种修饰有利于对转录因子和RNA聚合酶II的招募。另外一些组蛋白甲基化修饰则与沉默基因高度关联。例如，H3K9me3以及H3K27me3在哺乳动物细胞中发挥促进染色质凝集的作用(Nair et al., 2018)。

2.3 转录因子结合与染色质可及性

核小体的定位、解聚和重定位，以及组蛋白多种修饰方式，均与转录因子的结合有一定关联，从而影响基因的表达水平。有关转录因子如何取代核小体结合位置的推测很多。其中一种最简单的假设模型是：当核小体组蛋白发生解聚时，转录因子与组蛋白竞争性结合DNA位点，使该区域染色质的开放程度增强，从而为其它转录因子或辅因子的加入提供条件(Workman and Kingston, 1992; Svaren et al., 1994)。这种假设模型不涉及转录因子与核小体的直接作用，为一种被动的竞争模型(Workman and Kingston, 1992; Svaren et al., 1994)。理论上，这种模型更适用于解释常染色质区转录因子的结合模式，因为异染色质区核小体的周转速率明显不能给转录因子提供足够的机会去竞争结合核小体DNA (Bao et al., 2015; Swinstead et al., 2016)。另一种有染色质重塑复合体参与的核小体解聚过程对染色质开放和转录因子的结合更为重要。该过程可通过顺式调控方式进行，即转录因子首先与核小体间的linker DNA结合，进一步通过竞争性结合或在重塑复合物帮助下使邻近核小体解聚，从而建立该区域的染色质可及性(Mirny, 2010)。反式染色质重塑过程涉及远端一段被转录因子结合的开放染色质区，该区域在其它转录因子或者辅因子的帮助下，使目标染色质区域的核小体被逐出，从而发生染色质重塑(Almer et al., 1986; Taberlay et al., 2011)，此机制类似远端增强子的调控。

3 染色质可及性与植物生长发育和环境的响应

近几年，植物领域有关染色质可及性检测方法的应用、全基因组调控元件的挖掘以及染色质可及性参与植物生长发育和环境响应的研究相继被报道。现将近几年的相关研究进展总结如下。

3.1 植物领域染色质可及性检测方法的应用与发展

植物领域有关染色质可及性研究在早期也是采取Southern blot方法进行。日本科学家Kodama等(2007)报道了拟南芥5号染色体上一段480 kb区间染色质的DNase I超敏感位点。而全基因组水平染色质可及性检测最早是在2012年，由美国威斯康星大学Jiang团队以水稻(*Oryza sativa*)和拟南芥为材料进行研究(Zhang et al., 2012a, 2012b)。该团队采用的DNase-seq方法是由Boyle等(2008)开发的末端捕获法发展而来。2018年和2019年，该团队相继报道了染色质可及性在玉米(*Zea mays*)和马铃薯(*Solanum tuberosum*)等农作物基因组上的分布特征(Zhao et al., 2018a; Zeng et al., 2019)。Sullivan等(2014)利用DNase-seq双酶切法构建了拟南芥全基因组调控元件和转录因子结合图谱，并对光照以及黑暗条件下染色质可及性的动态变化进行了分析。Lu等(2017)将流式细胞分选技术与ATAC-seq方法相结合，排除了细胞器DNA对染色质可及性分析的干扰。Zhao等(2020)将MNase-seq方法应用到拟南芥染色质可及性检测上，发现了一些DNase-seq方法未检测到的开放染色质位点。到目前为止，DNase-seq、ATAC-seq和MNase-seq等常用的染色质可及性检测方法均已转化应用到植物领域。

3.2 植物基因组开放染色质区域特征

根据已知的染色质可及性影响因素，目前植物领域有关开放染色质的特征描述主要集中在全基因组分布特征分析、与转录因子结合的关联分析和与组蛋白修饰的相关分析等方面。首先，整体而言，植物基因组中开放染色质的分布与酵母、线虫(*Caenorhabditis elegans*)和人类细胞中相似，主要分布于常染色质区，少见于异染色质区。研究人员进一步将开放染色质按照其相对编码基因的位置进行分类，发现大多数开放染色质位于基因附近上下游1–2 kb范围内，其余部分位于基因间区；且开放染色质的分布比例与基因组大小有一定关联。例如，人类细胞中约39%的开放染色质分布于内含子区域(Thurman et al., 2012)，而拟南芥和水稻等植物基因组较小，仅分别检测到5%和11%的开放染色质位于内含子区(Zhang et al., 2012a, 2012b)。与此类似，植物基因组越大，在其上检测到位于基因间区的开放染色质比例也越高(Zhang et al., 2012a, 2012b)。其次，染色质可及性数据可以很好地覆盖转录因子和其它调控蛋白的ChIP-seq数据结果。Zhang等(2012a)构建的拟南芥花蕾开放染色质图谱分别覆盖了花发育关键转录因子AP1 (APETALA1)和SEP3 (SEPALLATA3)的ChIP-seq数据结果的95%和90%。再次，研究发现开放染色质区边界处往往有固定结合的核小体，其上的组蛋白修饰标记对该区域基因的表达有重要调节作用。水稻基因间区开放染色质与H3K27me3有明显的关联，而H3K27me3是一类被多梳蛋白复合体(polycomb protein complexes)控制的抑制型组蛋白标记，在拟南芥中发现其可抑制大量基因的表达(Zhang et al., 2007)。相反，一些活跃表达基因的近端会富集一些其它组蛋白标记。例如，H3K4me3出现在转录起始位点附近，而H3K4me1和H3K36me3在基因区分布较多。也有研究认为，具有H3K9ac和H3K27ac标记的基因间区开放染色质很可能是一些潜在的增强子元件(Lu et al., 2019)。

3.3 染色质可及性与植物生长发育

植物生长发育涉及一系列组织和时期特异基因的表达，转录因子与调控元件的适时结合是发育过程正常进行的关键开关。真核生物中1个转录因子可与多个调控元件结合从而调控多个基因的表达；同时，1个调控元件也可被多个转录因子调节。因此，植物在发育过程中的基因表达调控网络错综复杂，传统的ChIP-seq技术只能对某1个或某几个特定的转录因子结合位点进行分析，而以DNase-seq为代表的染色质可及性检测技术则可以一次性获得某类组织或特定发育时期所有开放染色质信息，从而实现全基因组范围内的调控元件挖掘分析。结合RNA-seq和ChIP-seq等数据，可有效获得该发育时期或组织中的特异性调控元件及基因表达调控网络关系。例如，Pajoro等(2014)利用DNase-seq方法检测到拟南芥花发育过程中染色质的动态变化，并结合基因表达数据构建了花发育时期特异的基因表达调控网络，同时发现转录因子AP1和SEP3可能参与染色质可及性调节。该类技术方法不仅可应用于模式植物拟南芥，也可应用于作物。例如, Qiu等(2016)利用DNase-seq技术检测了番茄(*Lycopersicon esculentum*)果实发育过程中的DNase I敏感位点，获得了一系列与番茄果实成熟相关的潜在调控元件，同时发现高转录活性的基因附近开放染色质区域往往被H3K4me组蛋白标记。随后，又有研究人员利用MNase-seq方法探究组蛋白变体与染色质可及性的关系。Dai等(2017)利用MNase-seq方法证实，在拟南芥花发育过程中, SWR1复合物通过介导组蛋白变体H2A.Z在启动子和增强子区域–1/+1核小体位置沉积，进而调节该区域的染色质可及性，抑制或激活基因表达，从而参与花序形态建成、花青素合成、大孢子母细胞特化、减数分裂、雌配子体发育以及植物免疫应答等过程(Qin et al., 2014; Cai et al., 2017, 2019, 2021a, 2021b, 2021c; Zhao et al., 2018b)。将染色质可及性检测技术应用于特定细胞类型上，可进一步加深人们对某一类型细胞中基因调控网络的精确认识。Sijacic等(2018)结合ATAC-seq和INTACT (isolation of nuclei tagged in specific cell types)方法分别获得拟南芥茎尖分生组织及已分化叶肉(两种特异类型)细胞的全基因组开放染色质图谱，并通过定量比较分析找出了细胞类型特异的开放染色质区域，及通过motif分析构建了细胞类型特异的转录因子调控网络。

3.4 染色质可及性与植物的环境响应

作为固着生长的生物，植物经常会面临多种多样的生物和非生物胁迫，因此，在长期进化过程中植物形成了许多响应机制。研究发现，一些转录因子和蛋白激

酶等参与植物的抗性和逆境响应; 同时, 越来越多的证据表明, 表观遗传修饰和染色质重塑等过程在逆境响应基因的表达过程中起重要调节作用。故研究人员推测环境因素可能通过诱导植物染色质的结构改变从而影响调节蛋白与调控元件的结合, 进而影响相关基因的表达。染色质可及性技术的出现为全基因组水平染色质动态变化检测及关键逆境响应调控元件的挖掘提供了可能。Sullivan等(2014)利用DNase-seq方法在拟南芥中构建了高分辨率的开放染色质图谱, 并通过比较分析建立了拟南芥响应光照和高温条件的转录因子与调控元件结合的动态调控网络。Jégu等(2017)采用ATAC-seq方法揭示了SWI/SNF类染色质重塑复合物蛋白BAF60通过调节染色质可及性参与拟南芥幼苗响应光周期的分子机制。进一步将染色质可及性研究与组蛋白修饰分析相结合, 可获得更丰富的调控信息。Zeng等(2019)利用DNase-seq方法在马铃薯块茎中鉴定到大量响应冷胁迫的开放染色质及潜在调控元件, 并发现大部分冷响应基因区同时富含H3K4me3和H3K27me3两种组蛋白修饰标记。此外, 与进化相结合的研究可以使人们更深刻地理解调控序列在不同物种间的进化关系。Han等(2020)利用DNase-seq方法在二穗短柄草(*Brachypodium distachyon*)、粟(*Setaria italica* var. *germanica*)和高粱(*Sorghum bicolor*) 3个近缘禾本科植物中鉴定到一系列受冷诱导的开放染色质区域, 并对其序列的进化关系进行了分析。此外, 对不同环境条件下开放染色质的比较分析发现, 植物在响应不同环境条件时存在染色质动态变化差异。Raxwal等(2020)利用FAIRE-seq和DNase-seq技术获得了拟南芥幼苗在响应热、冷、盐和干旱等胁迫条件时的开放染色质信息, 发现4种环境胁迫中, 相较于盐和干旱, 冷、热更明显地促进染色质开放。同样, 分离特定的细胞类型可精准分析该类细胞中染色质可及性受环境条件的影响。Tian等(2021)将ATAC-seq和INTACT方法相结合, 解析了拟南芥叶片维管组织和表皮细胞在响应光周期过程中的不同染色质动态变化情况。

4 结语与展望

21世纪以来, 随着测序技术的飞速发展, 测序成本越来越低, 组学检测技术已成为一种十分常用的技术手段。DNase-seq、ATAC-seq和MNase-seq等核酸酶结合高通量测序方法的出现使全基因组范围内染色质可及性检测变得简便且高效。人们清晰地认识到基因组上染色质的开放程度并非均匀分布, 核小体定位也并非一成不变, 它们会随着时间和空间的变化发生动态调节。染色质动态变化过程涉及核小体重定位、组蛋白修饰调节及转录因子结合等。细胞在生长发育和环境响应过程中出现的染色质构象改变及动态平衡使得调控元件适时地与转录因子结合, 促进远端调控序列与靶基因互作。因此, 染色质动态调节与基因表达调控有密切联系。

近年来, DNase-seq和ATAC-seq技术已广泛应用于植物开放染色质检测, 是一种非常可靠的调控元件挖掘方法。Zhu等(2015)利用GUS基因报告系统在拟南芥中验证了14个经DNase-seq分析预测的潜在增强子元件, 其中10个(71%)被证明与预测结果一致。然而, 植物领域染色质可及性研究仍存在一个关键问题, 即大多数研究使用的实验材料是包含多种细胞类型的混合植物组织。某一调控元件可能仅在一类细胞中处于开放状态, 而在其它细胞类型中并不开放。当采用混合组织样品对其进行检测时, 我们获得的是该位点在所有细胞类型中的综合结果, 而一些细胞类型特异的重要开放位点会因此被掩盖掉。故在植物领域应用INTACT结合DNase-seq/ATAC-seq方法以及对单细胞或low-input检测方法的开发显得十分必要。其次, 随着高通量测序技术的发展, 10X genomics结合ATAC-seq的单细胞测序技术已商业化, 通过制备单细胞核悬液的ATAC-seq分析也是解决混合组织染色质可及性分析过程中细胞异质性问题的策略之一。再次, 染色质的高级结构在染色质调控方面同样发挥非常重要的作用, 然而植物中的相关报道仍十分有限。将DNase-seq或ATAC-seq技术与Hi-C (Lieberman-Aiden et al., 2009)或ChIA-PET (Fullwood et al., 2009)技术结合, 分析远端调控元件参与基因表达调控的机制, 将有利于人们更好地理解染色质空间结构对基因表达调控的影响。总之, 随着测序技术的不断发展, 人们对染色质动态调节参与基因表达调控的分子机制的认识会更加深入。

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Chromatin Accessibility and the Gene Expression Regulation in Plants

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Abstract The topological organization of nucleosomes across the genome is non-uniform. While densely arranged within constitutive heterochromatin, histones are depleted at regulatory loci. Chromatin accessibility is the degree to which nuclear macromolecules are able to physically contact with regulatory DNA. Following the development of next-generation sequencing technology, a variety of quantitative methods, including DNase-seq, ATAC-seq, MNase-seq and NOMe-seq, have been developed to measure genome-wide chromatin accessibility easily and efficiently. In this review, we first introduced the technical principles of the four principal methods for measuring chromatin accessibility. And then we summarized the critical biophysical determinants of chromatin accessibility, including nucleosome occupancy, histone modification and TFs combination. Finally, we described recent advances of chromatin regulation during development and stress responses in plants. Our goal is to provide a reference for researches about genome-wide chromatin accessibility mapping, identification of *cis*-regulatory elements, and the dissection of epigenetic and genetic regulatory networks.

Key words chromatin accessibility, histone modification, DNase-seq, ATAC-seq, regulatory elements

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