RNA聚合酶II启动子近端暂停的动态调控

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摘要 转录暂停是一种进化上保守的、从细菌到人类普遍存在的关键转录调控机制。在多数后生动物中, 转录起始后, RNA聚合酶II在发育和应激反应相关基因的启动子近端区域暂停, 一旦受到环境或发育信号的刺激, RNA聚合酶II从启动子近端向基因体释放, 进行有效延伸。RNA聚合酶II启动子近端暂停是转录的关键限速步骤之一, 并作为转录早期检验点确保转录进程的正确运作。转录暂停和延伸调控异常与肿瘤及多种人类疾病密切相关。本文系统地从小RNA聚合酶II启动子近端暂停的建立、维持和释放等方面综述了对于这一重要转录限速步骤的调控, 着重讨论了相分离通过浓缩和传递转录相关因子在调控Pol II暂停和延伸过程中的作用, 并展望了针对转录暂停和释放这一过程设计靶向性治疗策略的潜力。

关键词 RNA聚合酶II, 启动子近端暂停, 转录延伸, 超级转录延伸复合物, 相分离

越来越多的研究发现, 在真核生物转录过程中, RNA聚合酶II(polymerase II, Pol II)受到高度复杂且精细的调控。对于绝大多数发育和应激反应相关基因来说, 转录的关键限速步骤发生在早期延伸阶段, 即转录起始后, Pol II并不直接进入有效延伸阶段, 而是暂停在转录起始位点(transcription start site, TSS)的近端区域, 等待进一步的释放信号^[1,2]。Pol II启动子近端暂停(promoter-proximal pausing)发生在TSS下游大约30~120个核苷酸处^[3]。作为转录的关键限速步骤和早期重要检验点(check point), 转录暂停可确保包括Pol II在内的转录机器的正确修饰和转录本5'端加帽的完成, 并在此基础上为后续转录激活提供可能^[4,5]。

Pol II暂停在启动子近端区域, 依赖于可准确募集通用转录因子(general transcription factor, GTF)和Pol II的核心启动子元件^[6]。暂停Pol II的稳定性由启动子相关转录因子、负性延伸因子(negative elongation factor,

NELF)和DRB(5,6-dichloro-1-β-D-ribofuranosylbenzimidazole)敏感性诱导因子(DRB sensitivity inducing factor, DSIF)调控^[7-9]。而Pol II由暂停释放进入持续有效延伸过程需要正性转录因子b(positive transcription elongation factor b, P-TEFb)复合物的介导。P-TEFb具有激酶活性, 包含催化亚基细胞周期依赖性激酶9(cyclin dependent kinase 9, CDK9)和调节亚基细胞周期蛋白T1/2(cyclin T1/2, CCNT1/2)^[10,11]。许多研究表明, P-TEFb是早期延伸阶段的关键因子, 其本身活性受到高度调节。P-TEFb主要以激活型和抑制型两种状态存在于3种复合物中: 超级转录延伸复合物(super elongation complex, SEC)家族成员^[12,13]、溴结构域蛋白4(bromodomain-containing protein 4, BRD4)-P-TEFb复合物^[14,15]和抑制型的7SK核内小核糖核蛋白(7SK small nuclear ribonucleoprotein, 7SK snRNP)-P-TEFb复合物^[16,17]。P-TEFb通过与转录因子和辅因子相互作用,

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直接或间接地被募集到启动子附近, 磷酸化Pol II羧基末端结构域(C-terminal domain, CTD)重复多肽序列上第二位的丝氨酸(Ser-2)、NELF和DSIF。NELF被磷酸化之后从Pol II上解离, 而DSIF被磷酸化后则对转录延伸起促进作用(图1)^[18,19]。此外, Pol II从启动子近端暂停区域释放进入持续延伸阶段, 还需要许多其他类型因子的协同作用, 改变染色质的状态, 调节新生RNA的加工, 帮助Pol II维持其持续合成能力^[20]。目前认为, 转录暂停的程度取决于如何平衡暂停因子(如NELF、DSIF)和包括激活型P-TEFb在内的激活因子在暂停区域的募集。

对细胞内众多的生化反应有序而高效的调控, 是确保细胞行使其正常功能的重要因素。就转录调控来说, 这一过程具有精确的时空性, 且所涉及的转录因子、辅因子等数目繁多。那么转录是如何快速响应细胞的生理与功能需求? 也就是说, 所需的转录机器是如何快速地组装起来? 最近使用超分辨率活细胞成像技术的研究显示, 通过蛋白质液-液相分离(liquid-liquid phase separation, LLPS), Pol II在细胞内会形成动态的簇集点或凝聚物^[21-25]。液-液相分离的发生依赖于蛋白质的固有无序区域(intrinsically disordered regions, IDRs), 且能进一步促进包含IDR的蛋白质在细胞内发生聚集^[26,27]。研究人员所观察到的这些Pol II凝聚物为转录的相分离模型提供了强有力的证据, 即在转录活跃位点蛋白质液-液相分离可起到快速且动态地浓缩和传递转录相关因子的作用。

本文将系统综述参与Pol II启动子近端暂停的建立、维持和释放各个过程的关键因子及其作用机制, 着重讨论相分离通过浓缩和传递转录因子在Pol II暂停、释放和延伸过程中起到的作用, 总结目前已发现的转录暂停和释放之间的平衡紊乱与人类疾病之间的联系, 并展望针对该阶段设计靶向性治疗策略的可能性。

1 Pol II启动子近端暂停

转录起始后, Pol II开始合成新生RNA。在新生RNA链尚不足10个核苷酸时, 与DNA模板链结合并不够紧密, 很容易从DNA模板链上脱落下来, 发生转录流产, 并重新启动转录起始; 当新生RNA链超过11个核苷酸之后, Pol II开始离开启动子区, 进入早期延伸阶段。在延伸过程Pol II也并非持续沿模板链前行合成RNA, 时常会出现暂停现象^[3]。Pol II在启动子近端暂

图1 (网络版彩色)转录起始、暂停和延伸。RNA Pol II在起始转录完成, 进入早期延伸阶段后, 在DSIF和NELF等因子的共同作用下, 会暂停于启动子近端。一旦受到环境或发育信号的刺激, 超级转录延伸复合物会被募集至该处, 促进Pol II向基因体释放, 进行有效转录延伸

Figure 1 (Color online) Transcription initiation, pausing and elongation. After transcription initiation and synthesis of a short nascent transcript, Pol II pauses at the promoter-proximal region, associated with NELF and DSIF. In response to developmental signals or environmental stress, SEC, one of the most active P-TEFb containing complexes, can trigger the release of paused Pol II into gene body

停的现象最早在果蝇中发现, 在非热休克条件下编码热休克蛋白70(Hsp70)的基因启动子近端-12~+65核苷酸之间积累了大量的Pol II^[28,29]。随后, 在原癌基因FOS及MYC上也发现启动子近端Pol II暂停的现象^[30,31]。随着染色质免疫共沉淀合并二代测序技术(chromatin immunoprecipitation sequencing, ChIP-seq)的成熟与广泛应用, 越来越多的证据显示Pol II启动子近端暂停在转录过程中非常普遍, 对于发育和应激反应相关基因的转录调控尤为重要^[32,33]。

1.1 Pol II启动子近端暂停的建立

Pol II在启动子近端的暂停发生于转录起始之后, 是基因表达调控的一个关键节点。对于其建立过程, 研究人员有着深入的研究。Pol II的转录从起始前复合物(pre-initiation complex, PIC)的组装开始。PIC包含Pol

II、通用转录因子和大量的共调节因子。其中通用转录因子II H(TFIID)的参与是PIC组装的最后步骤。TFIID是由大约10个亚基组成的复合物,不仅能够通过破坏碱基配对开放启动子,从而起始转录,还可以通过其激酶亚基细胞周期依赖性激酶7(cyclin dependent kinase 7, CDK7)磷酸化Pol II CTD重复多肽序列上第5和7位的丝氨酸(Ser-5、Ser-7)^[34-37]。Ser-5磷酸化是这两种修饰中研究得较多的一种,它发生在转录起始过程中,随着Pol II进入延伸阶段, Ser-5位点上逐渐发生去磷酸化^[38]。因此, Pol II CTD Ser-5磷酸化通常被看作暂停Pol II的标记。Ser-5被磷酸化的Pol II CTD不但能招募加帽酶,增强其活性,使其对新生RNA的5'端进行加帽修饰,还可以募集负性转录延伸因子DSIF和NELF,使得Pol II暂停在启动子近端区域^[39,40]。此外, Lin等人^[41]于2013年首次发现增强子与Pol II启动子近端暂停之间的重要联系。在小鼠胚胎干细胞中,转录因子ELL3结合在增强子上,广泛地调控谱系发育相关基因启动子近端Pol II暂停的建立,对于在干细胞分化过程中这类基因的激活起到关键作用。

1.2 Pol II启动子近端暂停的稳定性

在Pol II启动子近端暂停建立之后,对于不同的基因来说, Pol II的命运是不尽相同的,有可能会从模板链上脱落而导致提前终止,或快速进入有效转录延伸阶段,也有可能稳定地停滞于启动子近端区域。研究人员利用从中药雷公藤中提取的二萜类化合物雷公藤内酯醇(triptolide, TPL)对Pol II启动子近端暂停的稳定性进行了探索。TPL能抑制TFIID解旋酶亚基B组着色性干皮病偶联因子(xeroderma pigmentosum group B-complementing protein, XPB)的腺嘌呤核苷三磷酸(adenosine triphosphate, ATP)酶活性,从而阻止转录的重新启动^[42-44]。因此,在TPL处理之后的不同时间点测量Pol II在基因上的富集度,可探究这些基因上已经存在的暂停Pol II的状态。全基因组分析表明, Pol II在不同的基因启动子近端区域暂停的时间不同,在大多数基因上仅暂停几分钟,而在少数几个基因上可稳定暂停长达几小时^[45]。然而,这些研究依然不能清晰直观地反映暂停Pol II的稳定性及其动态转变过程。

蛋白质液-液相分离是一种物理化学过程,通过液-液相分离产生的凝聚物允许包括蛋白质在内的生物大分子在其相内迅速运动。最近所观察到的Pol II及各种转录相关因子以相分离形式形成凝聚物的现象^[23-25,46],

提示我们Pol II相关凝聚物的动力学变化特征可能会成为判断暂停Pol II稳定性的标准之一。

1.3 Pol II启动子近端暂停的维持

转录暂停后,可能被提前终止,也可能提前释放进入延伸阶段。根据目前的研究,主要从两方面来维持Pol II在启动子近端的暂停:阻止启动子近端转录的提前终止和防止暂停Pol II的提前释放。

体外转录延伸的研究发现,转录延伸抑制因子DSIF和NELF共同作用,将Pol II阻滞在启动子近端^[8,9]。DSIF由SPT4和SPT5亚基组成。类似于Pol II CTD, SPT5的羧基末端区域也存在一段重复区域,该区域可被TFIID的激酶亚基CDK7和P-TEFb中的CDK9磷酸化,并参与募集和激活新生RNA的加帽复合物^[47-50]。生化和结构生物学方面的研究表明, SPT5与上游DNA模板序列和新生RNA有直接接触,这可能有助于维持Pol II的暂停^[51-54]。NELF是一个四亚基复合物,由NELF-A、NELF-B、NELF-C(或异构体NELF-D)和NELF-E组成。在DSIF存在的情况下, NELF能够有效抑制体外转录^[9]。然而,细胞内敲除NELF通常会引起启动子近端Pol II水平的降低,并导致许多暂停基因转录水平的降低^[55,56]。NELF的所有4个亚基都能与Pol II以及RNA结合,可能增加了Pol II与新生RNA之间的结合力,有助于抑制早期转录终止^[54,57]。此外, Pol II在启动子近端暂停于启动子及其下游第一个核小体之间。最近的研究发现,破坏NELF复合物将导致Pol II离开暂停位点并向下游移位,可能造成转录提前终止,说明NELF在维持Pol II暂停在正确位置上的重要性^[58]。

维持Pol II在启动子近端暂停还需防止Pol II提前释放进入延伸阶段。在胚胎干细胞中,与暂停状态增强子相结合的ELL3调控了暂停Pol II在其相邻的发育相关基因启动子上的建立与维持。在胚胎干细胞分化过程中, ELL3则通过黏连蛋白/中介因子复合物(cohesin/mediator)介导的增强子——启动子相互作用,并进一步募集超级转录延伸复合物到启动子上,从而激活发育相关基因的转录^[41]。近些年的研究发现, Pol II相关因子1复合物(Pol II associated factor complex, PAF1C)等转录因子可通过抑制SEC的募集,来阻止暂停Pol II的释放,从而维持Pol II处于暂停状态^[59,60]。尽管其他研究得出了相反的结论,即这些因子通过招募SEC激活暂停Pol II的释放延伸^[61,62]。这些差异可能是由于PAF1C等因子对Pol II暂停的调控存在细胞或基因的差异性,

也可能是由于这些因子会与其他不同因子协同发挥着阻止暂停Pol II释放和后续促进Pol II释放的双重作用。

1.4 Pol II启动子近端暂停释放

许多转录相关因子在促进暂停Pol II的释放中发挥着重要作用。MYC可以促进Pol II从启动子近端暂停的状态中释放,而锌指蛋白ZFP281(zinc finger protein 281)则可特异地将MYC募集至靶基因启动子之上^[63,64]。此外,除了对于Pol II启动子近端暂停的建立起到重要作用之外,研究表明,增强子也参与Pol II从暂停到释放阶段的调控。BRD4与其相互作用因子JMJD6(Jumonji domain-containing protein 6)与增强子结合,远程促进Pol II启动子近端暂停释放^[65]。PAF1则能抑制增强子活性,阻碍Pol II从近端暂停状态的释放^[60]。启动子近端暂停的Pol II能否释放进入有效延伸状态,受到诸多因素的综合调控,包括负性延伸因子的失活或驱离、促进染色质开放的表观遗传机器的招募,以及将P-TEFb从其抑制型复合物中释放出来^[66,67]。这些过程有助于为Pol II的转录进程清除障碍,并募集更多的延伸相关因子。

2 Pol II启动子近端暂停释放的调控机制

当受到环境或发育信号的刺激后,Pol II从启动子近端暂停的位置逃脱,向基因体前行,进行有效地转录延伸,以响应细胞及机体的需求。P-TEFb复合物的活性对于转录从暂停到延伸的调控起到至关重要的作用。最新的研究表明,相分离介导了P-TEFb从抑制型向激活型复合物转变的过程^[68,69]。

2.1 P-TEFb复合物的多样性

早在1976年,Sehgal等人^[70]发现,使用ATP类似物DRB处理人宫颈癌HeLa细胞会导致全长mRNA的转录水平显著下降,而大量生成已加帽的转录本短片段。随后Handa研究团队^[8,9]在HeLa核抽提物中鉴定出来了介导DRB在细胞内抑制转录延伸的两个因子DSIF和NELF。而Marshall和Price^[71]则发现DRB在细胞内靶向P-TEFb,抑制其活性。

传统的生化研究发现,P-TEFb主要存在于7SK snRNP-P-TEFb、BRD4-P-TEFb和SEC家族复合物这3种大分子复合物中^[16,72,73]。细胞中超过90%的P-TEFb被限制在抑制型7SK snRNP-P-TEFb复合物中,处于催化失活状态^[19]。该复合物中的六亚甲基双乙酰胺诱导蛋

白1(hexamethylene bis-acetamide-inducible protein 1, HEXIM1)或HEXIM2二聚体蛋白直接结合P-TEFb,发挥抑制作用^[74]。当基因激活,尤其在基因快速诱导活化时,P-TEFb从抑制型复合物中释放进入SEC中,或与BRD4相互作用,发挥激酶活性^[14,19]。虽然BRD4-P-TEFb和SEC都能够催化Pol II CTD Ser-2的磷酸化,但是仍然需要阐明这两种不同复合物如何协同发挥促进转录延伸的作用。研究显示,在人类免疫缺陷病毒(human immune-deficiency virus, HIV)基因在宿主体内的转录调控中,BRD4仅能维持HIV本底水平的转录,而病毒蛋白转录反式激活因子(trans-activator of transcription, Tat)募集SEC是HIV完全被转录激活所必需的^[75,76]。在胚胎干细胞向神经谱系定向分化过程中,SEC亦介导了视黄酸(retinoic acid, RA)诱导的同源盒基因(homeobox genes, HOX)等基因的快速转录激活^[77]。

2.2 抑制型与激活型P-TEFb复合物转变控制转录释放

许多研究表明,7SK snRNP及其相关因子能够被招募到启动子近端区域,通过结合并抑制P-TEFb,阻止暂停Pol II的释放。例如,全基因组研究显示,HEXIM1作为抑制型P-TEFb复合物的组分之一,广泛结合在基因启动子处^[78]。目前发现的能够募集7SK snRNP到启动子近端区域的因子包括HIV转录因子Tat、磷酸酶PPM1G、剪接因子SR蛋白和转录中介因子KAP1等^[79-82]。抑制型的P-TEFb被募集到Pol II暂停区域,从而使得激活型转录相关因子有可能在附近捕获P-TEFb,促进转录进入延伸阶段^[79-82]。然而,P-TEFb从抑制型到激活型转变的机制仍然是一个悬而未决的问题。最近的研究提出,SEC的组分可能通过蛋白质液-液相分离的作用将P-TEFb从抑制型复合物中萃取出来,转变成具有激酶活性的SEC液滴,发挥转录激活和延伸作用^[68]。

2.3 相分离在转录调控中的作用

弱的、多价的蛋白质相互作用被认为是相分离的主要驱动因素之一^[83]。虽然蛋白质内的低复杂性结构域(low complexity sequence domain, LCD)或IDR缺乏固定的三维结构,但是此类结构域通常包含重复元件,可以作为电荷或疏水键多价相互作用的基础^[84]。转录因子通常由DNA结合结构域(DNA binding domain, DBD)和激活结构域(activation domain, AD)组成。一般

来说, DBD具有良好的结构特征, 而AD则富含低复杂性的无序区. 转录因子可通过其AD中的无序区形成凝聚物, 并与基因转录激活密切相关^[24]. 目前, 相分离模型被用来解释Pol II所介导的转录的自组织过程. 在该模型中, 转录因子(如序列特异性DNA结合因子FUS、EWS和TAF15)、辅因子(如BRD4和mediator)和Pol II通过液-液相分离的驱动可形成一个动态的启动子相关凝聚物, 并浓缩其他转录相关蛋白质, 支持转录起始和Pol II CTD的磷酸化^[23-25,85,86]. 而促进Pol II从启动子近端暂停释放的凝聚体中则包含P-TEFb复合物, 对转录机器进入基因体至关重要^[87]. 磷酸化的Pol II CTD, 转录延伸因子和共转录RNA加工因子共同组成基因体凝聚物, 推动转录持续进行(图2)^[68,69,87].

2.4 SEC相分离调控Pol II启动子近端暂停的释放

SEC是一个多亚基的大分子蛋白质复合物, 因其包含P-TEFb和Pol II延伸因子ELL家族成员这样两类作用机制不同的转录延伸因子, 被命名为超级转录延伸复合物^[73]. SEC最早是在2010年由Lin等人^[12]通过生物化学的方法分离纯化在急性白血病中因11q23染色体易位形成的MLL(mixed lineage leukemia)融合蛋白而得到, 并鉴定出来的. 随后, 通过纯化HIV病毒蛋白Tat也得到了SEC^[12,76,88]. 除P-TEFb和ELL家族成员外, SEC还含有AF4/FMR2家族成员1/4(AFF1/4)、YEATS结构域蛋白家族成员ENL(eleven nineteen leukemia)或AF9,

以及ELL结合因子EAF1/2. 其中AFF1/4作为SEC的支架组分, 含有P-TEFb、ELL、AF9/ENL的直接结合位点. 目前的研究发现, SEC对于快速启动暂停Pol II的释放和持续延伸十分重要. 体外Pol II CTD磷酸化实验显示, 在目前已发现的P-TEFb复合物中SEC具有最强的激酶活性. 许多发育相关及应激基因的快速诱导, 以及疾病相关基因如原癌基因MYC的转录依赖于SEC. 因此, SEC在机体发育及疾病发生过程中的作用至关重要.

研究发现, P-TEFb可以相分离的方式溶入SEC液滴里, 发挥激酶活性磷酸化Pol II CTD, 促进转录暂停释放进入延伸阶段, 行使快速转录激活功能^[68,69]. 不同的SEC组分在体内具有不同的相分离能力, 而SEC液滴的流动性则需要多个SEC亚基共同发挥作用. SEC组分AFF4和ENL的IDR, 可通过疏水和静电作用在体外形成相分离液滴^[68]. ENL和AFF4可以作为支架, 通过液-液相分离动态地从抑制型HEXIM1-P-TEFb复合物中分离和浓缩出SEC的激酶模块P-TEFb, 从而促进SEC的快速组装(图3)^[68]. 破坏SEC液滴的形成显著降低转录本的持续合成效率, 致使细胞无法作出应激响应. ENL-IDR缺失突变体无法恢复ENL基因敲除引起的即早基因FOS的快速转录诱导缺陷^[68].

2.5 SEC复合物家族其他成员

哺乳动物中, SEC组分AFF1和AFF4属于AFF蛋白

图2 (网络版彩色)相分离与转录调控. RNA Pol II在细胞内会形成动态的相分离簇集点或凝聚物, 调控转录起始与延伸
Figure 2 (Color online) Phase separation and transcriptional regulation. RNA Pol II and its regulatory factors can form dynamic phase separated

图3 (网络版彩色)相分离调控Pol II启动子近端暂停的释放。在Pol II暂停释放过程中, SEC的组分ENL可通过LLPS方式将P-TEFb从抑制型7SK/HEXIM1复合物中萃取出来, 融合进入具有激酶活性的SEC液滴, 发挥快速转录激活作用

Figure 3 (Color online) Phase separation and release of promoter-proximal paused Pol II. P-TEFb can be extracted from the inactive 7SK/HEXIM1 complex by the SEC core component ENL via LLPS, and thus incorporated into the SEC droplets to function in pause release and transcriptional activation

家族, 该家族还包括AFF2和AFF3两个成员。研究表明, 分别以AFF2和AFF3为支架可形成SEC类似复合物2和3(SEC-Like 2 and 3, SEC-L2/3)。与SEC类似, SEC-L2/3复合物还包含有P-TEFb和ENL, 属于激活型P-TEFb复合物, 和SEC一起被归于SEC复合物家族^[13,73]。基因组学分析则进一步表明, SEC复合物家族各个成员对于靶基因的调控具有特异性^[13]。例如, AFF3可直接调控基因组印记基因与X染色体失活相关基因的表达^[89-91]。近年的全基因组关联研究(genome wide association study, GWAS)表明不同SEC家族成员支架组分与多种人类疾病的相关性^[92]。因此, 对于SEC-L2和SEC-L3的具体作用机制值得进一步探索。

3 转录暂停、延伸与疾病

基因转录暂停和延伸过程涉及多种蛋白质分子之间的协同作用, 任意环节的异常都有可能引起疾病发生。因与MLL融合或获得功能性点突变而导致的SEC组分的异常稳定, 在白血病和先天性CHOPS综合征(认知障碍和粗犷面相、心脏缺陷、肥胖、肺部受累、身材矮小和骨骼发育不良)中起主导作用^[72,93,94]。最近研究提出, SEC组分ENL与MLL融合后, 可以极大促进SEC相分离, 从而促进癌基因快速转录并维持高表达状态^[68]。在肾母细胞瘤中, ENL YEATS结构域的突变能够促进ENL通过相分离发生自组装, 进而募集SEC促

进癌基因高表达, 并引起肿瘤发生^[95]。这些发现将为SEC相关疾病发病机制的探索及靶向药物筛选方案提供了新的方向与思路。

在急性白血病中, MYC等癌基因的异常表达受SEC调控^[72,96]。特异地抑制AFF4与P-TEFb结合的药物KL-1/2, 对于控制MYC相关的癌细胞具有很好的效果^[97]。BRD4抑制剂JQ1也能够抑制MYC等癌基因的转录, 因而被认为是治疗癌症的潜在有效药物^[98,99]。有意思的是, SEC和感染性病毒HIV和人类乙型肝炎病毒(hepatitis B virus, HBV)在宿主体内的表达及复制密切相关;但对于HIV和HBV感染的患者, JQ1会导致HIV和HBV的重新激活^[100,101]。因此, 有必要针对这些转录延伸因子在调控致病基因方面的机制作深入详尽的探究, 为精准用药夯实基础。

此外, SEC-L2的支架组分AFF2被发现与脆性X智力低下综合征有关^[102], 而I型糖尿病、类风湿性关节炎和系统性红斑狼疮等自身免疫性疾病与AFF3的SNP高度相关^[103,104]。系统研究SEC家族成员在转录调控中的作用, 探索其异常在疾病发生发展中的机理, 将有助于寻找特异性的靶向治疗手段。

4 结语

基因表达调控是一个高度复杂的过程, 充分理解转录暂停、释放和快速延伸调控过程十分具有挑战性。相分离模型在一定程度上帮助我们理解细胞是如何快速改变其自身的基因表达程序。例如, 该模型解释了不同的转录相关因子是如何招募包括RNA Pol II在内的转录机器, 并平衡启动子近端暂停和延伸过程^[46,105]。转录凝聚物的高度动态性确保了高浓度延伸因子的聚集, 催化反应的高效率, 转录延伸的快速发生, 这样才使得转录过程能够响应各发育阶段不同的信号刺激^[106,107]。此外, 翻译后修饰如磷酸化、甲基化、乙酰化或泛素化也能够控制转录液滴的形成和动力学特性, 通过改变靶蛋白的相分离特性调节其功能活性^[108,109]。

目前主要是通过基因敲除的方法, 以及利用针对转录相关因子特异的小分子抑制剂, 来研究与启动子近端暂停及延伸相关的因子或大分子复合物在转录调控中的主要功能。阐明多因子在启动子近端暂停和延伸中的协同作用, 以及它们参与的各种细胞调节过程, 对于了解相关的人类疾病十分关键。因此, 从相分离的角度理解启动子近端暂停和延伸过程中大分子蛋白质复合物的凝聚与组装, 以及阐明相关复合物转

录调控的分子机制, 不仅有助于我们理解错综复杂的基因表达程序, 还可以为因启动子近端暂停和延伸过程异常而导致的人类疾病提供潜在的靶向精准治疗策略.

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Summary for “RNA聚合酶II启动子近端暂停的动态调控”

Dynamic regulation of promoter-proximal RNA polymerase II pausing

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Transcriptional pausing is a widely used regulatory mechanism to control precise gene expression from bacteria to humans. In metazoans, transcription initiation requires the recruitment of general transcription factors and RNA polymerase II (Pol II) to gene promoters. After it initiates transcription and synthesizes a short RNA, Pol II pauses at the promoter-proximal region, standing by for further cues to enter the productive elongation stage. Promoter-proximal Pol II pausing is one of the rate-limiting steps during transcription, functioning as an early transcriptional elongation check point, to ensure the correct modification of transcription machinery and the addition of the 7-methylguanosine cap to nascent transcripts.

Pol II pausing at promoter-proximal region is stabilized by the negative elongation factor (NELF) and the 5,6-dichloro-1- β -d-ribofuranosylbenzimidazole (DRB) sensitivity-inducing factor (DSIF). In response to developmental signals or environmental stress, the release of paused Pol II can be triggered by the positive transcription elongation factor b (P-TEFb), which is composed of the kinase CDK9 and its regulatory subunit CCNT1/2. The kinase activity of P-TEFb towards Pol II carboxy-terminal domain (CTD), NELF and DSIF is critical for P-TEFb to execute its function in pause release, and is thus tightly controlled. To date, it has been identified that P-TEFb mainly exists in three different complexes: the super elongation complex (SEC), BRD4-P-TEFb, and the inactive 7SK snRNP-P-TEFb complex. SEC, one of the most active P-TEFb containing complexes, is required for rapid gene expression in response to stress or developmental signals. The active and inactive forms of P-TEFb complexes co-exist *in vivo*, reaching equilibrium to meet the transcriptional needs of cells. The transition of Pol II from pause to productive elongation stage is achieved by dynamic exchange between the pausing factors and the positive elongation factors at the proximal promoter region.

Liquid-liquid phase separation (LLPS) in living cells is a physicochemical process that confines biochemical reactions within membrane-less compartments. Low complexity intrinsically disordered regions (IDRs) within proteins can initiate and promote phase separation and formation of liquid droplets, which in turn facilitate the multivalent weak interactions among these IDR-containing proteins. Recent studies using super-resolution live cell imaging technology have revealed that RNA Pol II can form dynamic foci in cells through LLPS and transitions between different phases, which are likely mediated by phosphorylated CTD. Moreover, factors involved in various transcription steps can rapidly and dynamically concentrate and exchange at the hot regulatory genomic loci. For example, SEC components are able to compartmentalize and concentrate P-TEFb from its inactive partners. Upon serum stimulation, SEC assembles promptly into phase-separated droplets at the immediate early genes (IEG), triggering rapid transcription induction.

Mutations in transcription regulators can lead to developmental defects and many human diseases, including cancers. For instance, abnormal stabilization of SEC components via MLL fusion plays a contributing role in leukemogenesis. Fusion of the SEC core component ENL with MLL can greatly enhance phase separation of SEC, through which the leukemic genes might be mis-activated. In addition, there is growing evidence that disease related mutations in transcriptional regulators can cause malfunction through aberrant condensation, providing potential targets for designing therapeutic strategies through phase separation interventions.

RNA polymerase II, promoter-proximal pausing, transcription elongation, super elongation complex (SEC), phase separation

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