纺锤体组装检验点: 染色体稳定性的守护神

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摘要 有丝分裂是真核生物进行细胞增殖的基本方式, 其根本目的是准确无误地将复制好的染色体平均分配到两个子细胞中。在细胞有丝分裂过程中, 纺锤体组装检验点的作用是产生“等待”信号, 直至所有的染色体都排列到赤道板上并建立正确的双极定向, 以确保染色体的均等分配。在高等动物中, 细胞的纺锤体组装检验点功能行使异常, 染色体分离将出现错误, 导致子代细胞的染色体数量不稳定, 进而诱发肿瘤或导致其他疾病的发生。纺锤体组装检验点一直以来都是细胞生物学家研究的热点, 然而其作用的分子基础和调控因素还不是十分明了, 该文将对近年来关于纺锤体检验点的研究进展进行总结和探讨。

关键词 有丝分裂; 动点; 纺锤体组装检验点; 染色体不稳定性; 肿瘤

Spindle Assembly Checkpoint: Guardian of Chromosome Stability

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Abstract The eukaryotic life stems from cell division by which parental genome is segregated into two daughter cells. To achieve the equal distribution, protein machinery named spindle assembly checkpoint (SAC) ensures all chromosomes bi-oriented and accurately attached to spindle microtubule prior to anaphase initiation. Perturbation of SAC assembly dynamics and functional plasticity contribute to chromosome instability and aneuploidy that contribute to tumorigenesis. This essay highlights recent progresses towards a better understanding of SAC. We also discuss the perspectives of future research on this exciting avenue.

Key words mitosis; kinetochore; spindle assembly checkpoint; chromosome instability; tumor

1 引言

在细胞有丝分裂过程中, 为了保证姐妹染色单体能够均等地分配到子代细胞中, 所有的染色体需要完成向细胞赤道板的排列并且建立正确的动点–微管连接。在此过程中, 纺锤体组装检验点(spindle assembly checkpoint, SAC)发挥着监督机能, 能够抑制后期的开始直至所有的染色体完成排列和双极定向。如果在染色体没有完全排列之前, SAC非正常失活导致细胞提前进入后期, 未排列到赤道板的染色体(含两条姐妹染色单体)将进入同一个子细胞中, 致使非整倍体细胞的产生。非整倍体会进一步促进染色体不稳定性(chromosome instability, CIN)的发生(图1)。CIN一直以来被认为是肿瘤发生发展的重要诱因, 而SAC相关基因的突变在多种肿瘤中均有报道[1], 所以研究SAC对于理解肿瘤的发生以及后续肿瘤的治疗具有重要的指导意义[2]。

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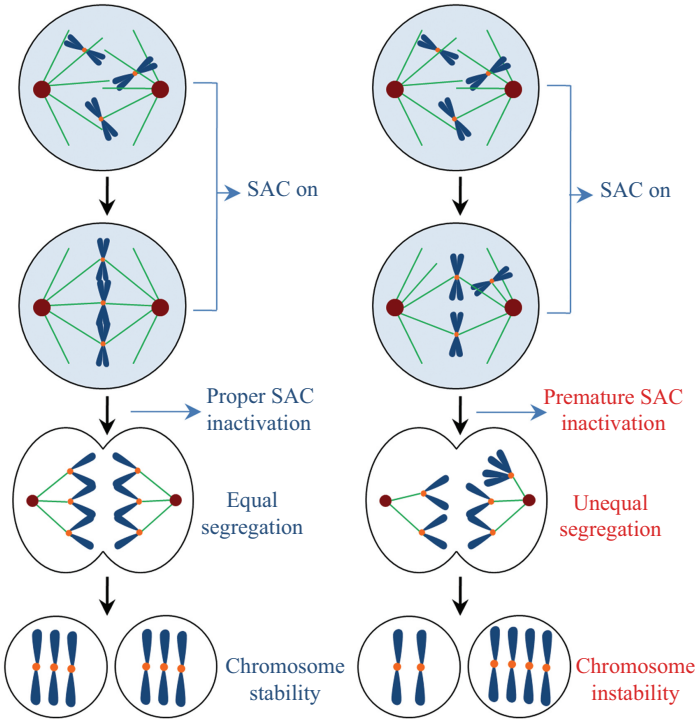


在正常细胞中(左侧), 当所有染色体都排列到细胞赤道板上并建立正确的动点–微管连接后才进入有丝分裂后期, 姐妹染色单体均等地分配到两个子代细胞中; 而在SAC异常的细胞中(右侧), SAC提前失活启动后期的开始, 还未排列好的染色体将不再分离即进入一个子细胞中, 导致两个子细胞中染色体数量不均等。

In normal mitotic cell (left), to ensure that sister chromosomes are distributed into daughter cells equally, anaphase onsets only when all chromosomes are aligned and proper kinetochore-microtubule attachments are established. In contrast, if SAC inactivates prematurely (right), misaligned chromosome will enter one daughter cell without segregation, leading to chromosome instability of progeny cells.

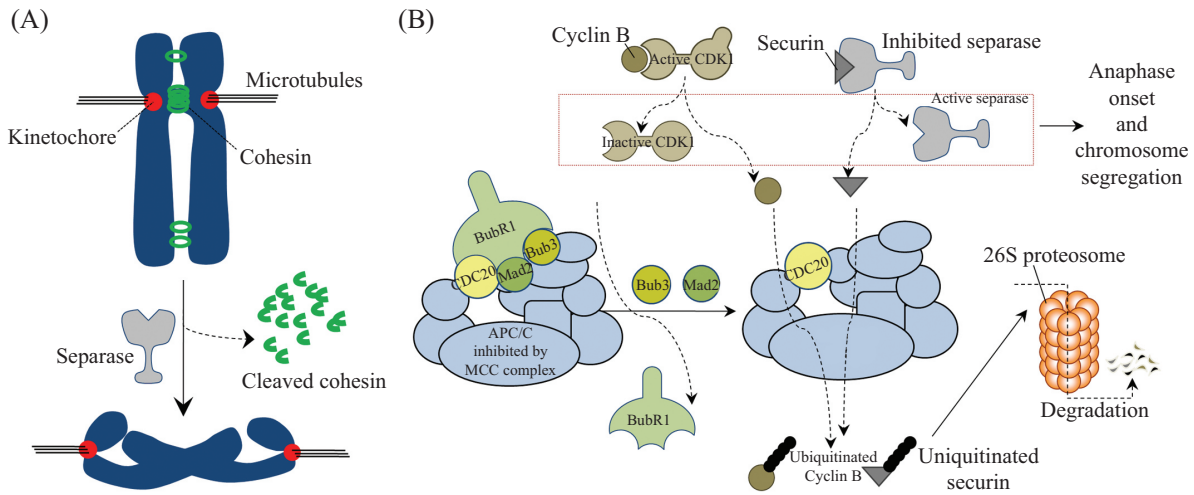
图1 SAC功能异常导致染色体不稳定性的模型

Fig.1 Model of chromosome instability induced by SAC defect

2 SAC的分子基础

有丝分裂后期的开始依赖于泛素连接酶APC/C(anaphase promoting complex/cyclosome)[3]介导的两个关键底物的泛素化降解: Cyclin B和Securin。其中, Cyclin B是有丝分裂期的一个主导激酶CDK1的激活因子, Cyclin B的降解导致CDK1激酶的失活; Securin是蛋白切割酶Separase的抑制蛋白。在有丝分裂的前中期, Separase处于被Securin抑制的状态, 当细胞进入后期时, APC/C介导Secrurin的降解从而释放Separase的活性, 激活的Separase对连接姐妹染色单体的Cohesin进行切割, 促进染色单体的分离(图2A)。而在前中期APC/C的活性受到SAC的抑制。SAC蛋白的基本成员包括Mad1、Mad2、Mad3/BubR1(酵母中为Mad3, 高等生物中进化为BubR1)、Bub1、Bub3和Mps1。其中, Mad2、Mad3/BubR1、Bub3以及CDC20组成有丝分裂检验点复合物(mitotic checkpoint complex, MCC)[4], MCC通过与APC/C的直接结合发挥抑制效果[5]。由于CDC20是泛素连接酶APC/C的激活蛋白, SAC通过Mad2、BubR1/Mad3和Bub3结合CDC20, 阻止CDC20对APC/C的激活, 达到抑制APC/C的效果, 阻止细胞提前进入后期[6]。当染色体完成排列并建立正确的微管连接后, SAC失活从而释放APC/C的活性, 进而介导Cyclin B和Securin的泛素化降解(图2B)。

SAC蛋白的六个成员以及CDC20在真核生物中具有很高的保守性, 说明SAC是真核生物在有丝分裂和减数分裂过程中保证细胞染色体稳定性所采用的共同机制。然而例外的是, 在果蝇细胞的有丝分裂和爪蟾卵母细胞的减数分裂过程中并不存在SAC[7-9], 在这两个分裂过程中保证染色体均等分配的机制还有待进一步的研究。在有丝分裂前期或前中期, SAC蛋白都定位在未被微管连接的或不正确连接的动点(也称作动粒)上, 然而它们在动点上的功能却迥然不同。本文侧重对近8年来哺乳动物中SAC的研究进展进行归纳总结和讨论。

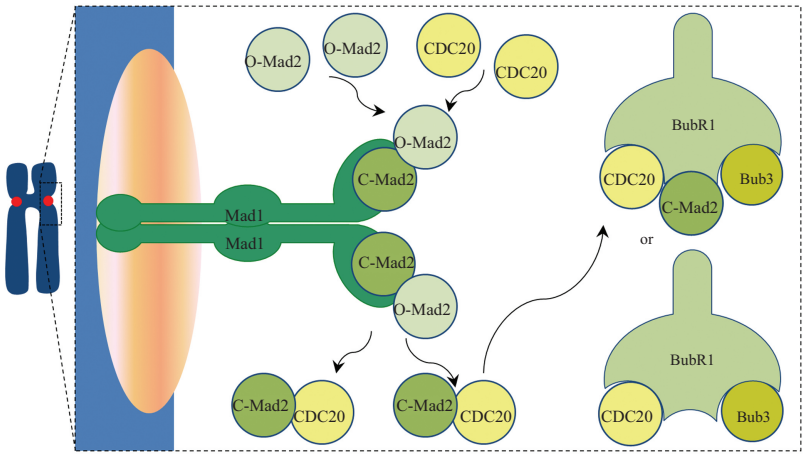


A: 在后期开始以前, 姐妹染色单体通过Cohesin蛋白连接在一起。后期开始时, 蛋白切割酶Separase切割Cohesin, 帮助姐妹染色单体分离; B: 当SAC失活, MCC复合物对APC/C的抑制解除后, APC/C将对CDK1激酶的激活蛋白Cyclin B以及Separase的抑制蛋白Securin进行泛素化, 介导其蛋白酶体的降解途径。CDK1激酶的失活和Separase的激活促进了后期的开始以及姐妹染色单体的分离。

A: when anaphase onsets, Cohesin which connects sister chromosomes will be cleaved by Separase, promoting sister chromosome segregation; B: SAC inactivation releases APC/C activity, which will lead to polyubiquitylation of Cyclin B (activator of CDK1 kinase) and Securin (inhibitor of Separase), leading to their degradation by 26S proteosome. CDK1 inactivation and Separase activation trigger anaphase onset and sister chromosome segregation.

图2 SAC失活启动后期以及染色体分离

Fig.2 SAC inactivation triggers anaphase entry and chromosome segregation



O-Mad2通过与结合在Mad1上的C-Mad2形成二聚体, 从而促进其与CDC20的结合, 结合CDC20的O-Mad2发生构象改变成为C-Mad2。CDC20-C-Mad2再与Bub3-BubR1结合形成四元复合物或者不包含Mad2的三元复合物。

Via binding to C-Mad2 bound on Mad1 dimer, O-Mad2 interacts with CDC20 and changes its conformation to C-Mad2. CDC20-C-Mad2 heterodimer and Bub3-BubR1 heterodimer then form a quaternary complex or ternary complex without Mad2.

图3 MCC复合物的形成过程

Fig.3 Formation of MCC complex

2.1 MCC复合物的形成

在有丝分裂期, SAC蛋白在动点上呈现高度的动态性, 然而MCC复合物抑制APC/C却不仅仅局限在动点上, 所以动点被认为是催化MCC复合物形成的平台。Mad2在细胞中存在两种构象, 根据结构上的差异将其区分为C-Mad2(closed Mad2)和O-Mad2(open Mad2)。C-Mad2与Mad1紧密结合并定位在动点上。 胞质中的O-Mad2与动点上的C-Mad2结合形成二聚体, 进而促进O-Mad2与CDC20的结合, 与CDC20结合后的O-Mad2将改变构象变成C-Mad2, 即Mad1-C-Mad2复合物催化O-Mad2与CDC20形成CDC20-C-Mad2复合物[10]。CDC20-C-Mad2进而结合BubR1/Mad3-Bub3复合物, 形成MCC四元复合物(图3)。

关于MCC抑制APC/C的作用机制尚存争议, 而争议的焦点主要集中在Mad2上。在MCC中, BubR1/Mad3和Mad2都可以结合CDC20, 一种观点认为, BubR1/Mad3通过KEN box与底物竞争结合CDC20, 达到抑制底物降解的作用[11], 而Mad2的作用被认为是稳定BubR1/Mad3与CDC20的结合[12-13]。另一种观点则认为, Mad2通过结合CDC20, 抑制CDC20与APC/C的结合, 也可达到抑制APC/C活性的作用[14], 在这一观点中BubR1/Mad3被认为负责稳定CDC20-Mad2之间的相互作用[15]。以上两种观点的前提是, 在MCC复合物中Mad2和其他蛋白的分子数量相当, 而结构生物学研究的结果表明, MCC复合物中Mad2、BubR1/Mad3和CDC20的比例确实为1:1:1[4]。然而另一研究结果表明, 在MCC复合物中, 相对于CDC20来说, Mad2的含量微乎其微, 但Mad2却是BubR1/Mad3结合CDC20所必需的[16]。最近的研究中, Han等[17]及Kulukian等[18]证明Mad2结合CDC20可以诱发CDC20构象的改变, 促进BubR1/Mad3对CDC20的结合, 其中C-Mad2的功能仅仅是催化CDC20-BubR1/Mad3复合物的形成, 并不参与MCC复合物的组成。

在MCC复合物组装过程中, 蛋白激酶Mps1也发挥着重要的作用。一方面, Mps1介导其他SAC蛋白的动点定位[19-24]; 另一方面, Mps1的激酶活性催化O-Mad2向C-Mad2的转变, 促进SAC蛋白之间的相互作用[19,25-27], 然而其中的机制还有待进一步的研究。Mps1对SAC蛋白的磷酸化修饰可能在MCC复合物形成过程中发挥重要作用[28]。虽然Mps1激酶的底物信息已有报道[29], 更多底物位点的鉴定和位点磷酸化的功能研究将对深入理解SAC起到至关重要的作用。Mad1的功能主要在于负责Mad2的动点招募, 然而最近的研究结果表明, Mad1除了招募Mad2外, 在SAC信号发生中还发挥着其他未知的作用[30-31]。

2.2 SAC蛋白动点定位的结构基础

SAC蛋白的动点定位机制一直以来都是有丝分裂领域的研究热点。如前所述, Mad1介导Mad2定位在动点上, 并且催化O-Mad2向C-Mad2的转变。通过将Mad1与组成型动点蛋白Mis12融合表达, 使Mad1持续定位在动点上, 即使在微管连接动点的状态下, Mis12-Mad1仍然能够介导中期细胞的阻断, 说明Mad1的动点定位是SAC发挥功能的关键[32]。然而, Mad1在动点上的受体蛋白还有待研究。虽然有结果表明, Hec1对Mad1的动点定位有帮助[33-35], 但是Hec1与Mad1之间的直接相互作用并未见报道。所以, Hec1介导Mad1的动点定位机制可能是间接作用。一个可能的中间承接者是Mps1, 因为Mps1在动点上的直接受体蛋白被证明是Hec1[36-38], 而Mps1是其他SAC蛋白动点定位所必需的。在酵母和线虫中, Bub1被证明是Mad1动点定位的直接结合蛋白[39-40]。并且在酵母中, Bub1与Mad1之间的相互作用依赖Mps1对Bub1的磷酸化以及Mad2的存在[39]。通过融合蛋白将酵母Mps1固定在动点上, Bub1可持续性地与动点结合, 但Mad1却仍然能够从动点上解离下来[21], 说明Mad1的动点定位还依赖于其他未知的蛋白。在人源细胞中, Bub1对Mad1的动点定位也是必需的, 然而体内和体外实验均未能证明Bub1和Mad1之间存在直接的相互作用, 并且Bub1的敲除仅仅导致Mad1的动点定位减少, 而不是完全消失[41], 说明在人源细胞中Mad1的动点定位同样也依赖于其他蛋白。Mps1是其中的一个潜在结合蛋白, 原因是Mps1帮助Mad1的动点定位, 然而似乎并不依赖于Mps1的激酶活性[19,25]。综上所述, Bub1和Mps1可能共同介导了Mad1的动点招募(图4)。此外, Plk1激酶介导的Mad1-T680磷酸化在Mad1的动点定位中也发挥着重要的作用[42]。

Bub1和BubR1在功能结构域上比较相似(图4A), 都有位于蛋白N-端的TPR结构域和结合Bub3的GLEBS区域以及蛋白C-端的激酶结构域[43]。酵母中BubR1的同源物Mad3并不含此结构域。Bub1和BubR1/Mad3以及Bub3在动点上的受体是KNL1[44]。Bub1-Bub3和BubR1/Mad3-Bub3通过两个方面与KNL1结合。首先, Mps1对KNL1的多个MELT序列进行磷酸化, 从而招募Bub3[22-24,45], 这也证实了之前关于Mps1的底物序列特征报道的正确性[44]。其次, Bub1和BubR1/Mad3通过TPR结构域与KNL1上面的KI序列结合[46-48]。虽然TPR-KI和MELT-Bub3协同增强Bub1和BubR1/Mad3与KNL1的相互作用[48], 然而破坏TPR-KI之间的相互作用既不影响Bub1和BubR1/Mad3的动点定位, 也不影响SAC的功能[49-50]。所以, TPR-KI结合的生理意义还有待进一步的研究。另外, Bub1和Bub3的动点定位是相互依赖的, Bub1在Bub3招募中的具体作用还不清楚, 可能的原因是Bub1通过对H2A-T120的磷酸化促进Aurora B激酶的着丝粒定位, 进而介导Mps1的动点定位, 激活Mps1-KNL1-

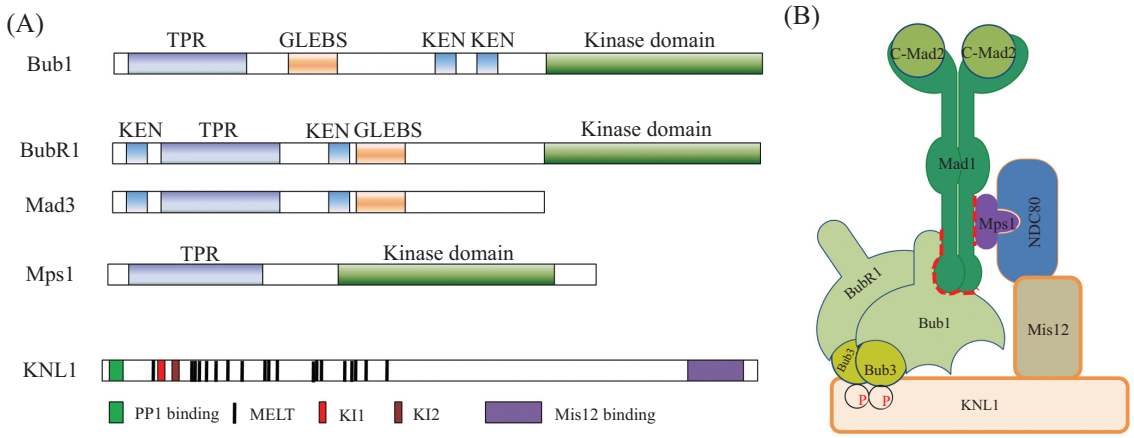
Bub3信号通路[37-38,51-52]; 此外, Bub1/Bub3复合物可以更加稳定地与KNL1结合。

如前所述, Aurora B激酶活性和Hec1共同介导了Mps1的动点定位[37-38]。有趣的是, Mps1本身的激酶活性也调节了自身的动点定位。当激酶活性受到抑制时, 其动点定位明显增强[25]。由于Mps1能够二聚并发生自磷酸化以增强自身的激酶活性[25,53], 所以Mps1可能通过自磷酸化调节自身在动点上的定位, 进而对SAC的活性进行控制。Mps1是一个关键的检验点激酶, 尽管在人类Mps1功能研究伊始就已经知道它的定位依赖于Hec1, 但详细的分子机制并不清楚。近来我们与国际同行共同揭示了Hec1的微管结合区域决定了Mps1的定位(图4B)。更重要的是, Aurora B激酶能够磷酸化Hec1, 进而促进Hec1与Mps1的结合[37]。

2.3 参与SAC功能的其他调节因子

除了六个SAC核心成员以及CDC20之外, 还有其他蛋白在SAC信号中发挥重要的作用。Aurora B激酶在有丝分裂期能够纠正错误的动点–微管连接[54], 从而产生未被微管连接的动点, 未被微管连接的动点将成为催化MCC复合物组装的平台, 这样说来Aurora B激酶在SAC方面也发挥了间接的作用。然而近年来, 越来越多的证据表明, Aurora B直接参与SAC功能的发挥[32,55], 其中最主要的功能就在于直接参与了SAC蛋白的动点定位, 而源头正是介导了Mps1与Hec1的直接结合[36-38,56]。

RNA相关蛋白PRP4(pre-messenger RNA processing 4)是一个在真核生物中保守的蛋白激酶, 人源的PRP4被发现定位在有丝分裂期染色体动点上。当细胞PRP4蛋白表达被抑制后, 微管解聚药物nocodazole处理并不能导致细胞的阻断, 说明PRP4敲除的细胞中SAC活性受到抑制。诚然, PRP4表达被抑制后, Mps1、Mad1以及Mad2的动点定位消失[57]。在酵母中核孔相关蛋白Xpo1p也定位在有丝分裂期的动点上, 并介导了Mad1的动点定位[58]。而在人体中, 同样是核孔复合物组分蛋白的Tpr是Mad1和Mad2的直接结合蛋白, 并且介导Mad1-Mad2在有丝分裂早前期核膜上面的定位[59]。Tpr的功能主要体现在两个方面, 即通过直接的相互作用维持了Mad1和Mad2的蛋白稳定性, 同时介导了Mad2的动点定



A: 人源Bub1、BubR1以及Mps1在结构上类似, 它们都含有N-端的TPR结构域和C-端的激酶结构域。不同的是, BubR1是否具有激酶活性尚存争议。Bub1和BubR1上的GLEBS结构域是Bub3的结合区段。酿酒酵母中与BubR1同源的Mad3没有激酶结构域。人源的KNL1结构示意图上显示有PP1的结合区域、Mis12复合物的结合区域、与Bub1的TPR结构结合的KI1序列、与BubR1的TPR结合的KI2序列以及19个MELT序列。B: SAC蛋白动点定位的示意图。Bub1-Bub3和BubR1-Bub3复合物结合在KNL1上面, 并且依赖于MELT序列的磷酸化。Mps1激酶通过NDC80复合物上面的Hec1定位在动点。而Mad1的动点定位则同时依赖于Bub1和Mps1, 图中红色虚线表示Bub1和Mps1与Mad1是否存在直接的蛋白相互作用尚不清楚。Mad2通过与Mad1结合定位在动点上。

A: human Bub1, BubR1 and Mps1 are structurally similar kinases with TPR domain at N terminus and kinase domain at C terminus. However, BubR1 is regarded as a psuedokinase, which is still debatable. Bub3 binds to the GLEBS domain on Bub1 and BubR1. Yeast Mad3, the homolog of human BubR1, lacks kinase domain. Human KNL1 is also shown with PP1 binding domain, Mis12 complex binding domain, Bub1-TPR binding KI1, BubR1-TPR binding KI2 and 19 MELT motifs. B: model for kinetochore localizations of SAC proteins. Bub1-Bub3 and BubR1-Bub3 localize to kinetochore via binding to phosphorylated MELT motif of KNL1. Hec1 recruits Mps1 by direct interaction. Both Bub1 and Mps1 are required for Mad1 recruitment, where red dashed lines represent uncertainty of direct interactions of Bub1-Mad1 and Mps1-Mad1. And Mad1 recruits Mad2.

图4 SAC相关蛋白的结构及动点定位示意图

Fig.4 Schematic shows of SAC related proteins and their kinetochore recruitments

位, 然而其中的分子机制还不清楚[59-60]。在Mad2的动点定位方面, 泛素连接酶Smurf2同Tpr一样, 既稳定了Mad2蛋白, 又参与介导Mad2的动点定位, 同样其中的机制还有待研究[61]。

凋亡相关蛋白TAp73在肺癌中低表达。有研究结果表明, TAp73与Bub1、BubR1以及Bub3直接相互作用, 并且介导了它们在动点上的定位, 提示肺癌细胞中的染色体不稳定性可能与TAp73低表达导致的SAC失活有关[62-63]。同样地, 人源蛋白RINGO C的敲除也影响到Bub1、BubR1等SAC蛋白的动点定位。RINGO C同Cyclin蛋白一样, 也是CDK激酶的激活蛋白, 然而RINGO C和Cyclin之间并没有序列同源性[64]。RINGO C是否通过调节CDK激酶活性来介导SAC蛋白定位还有待进一步的研究。前面提到Mps1对KNL1上面的MELT序列进行磷酸化, 提供了Bub3动点结合的位点, 然而肿瘤特异性的丙酮酸激酶PKM2在Bub3定位方面也发挥着重要的作用, PKM2通过磷酸化Bub3的Y207位对Bub3复合物的定位进行调控[65]。在最近的研究中, 一个有关人胶质瘤的新基因*BuGZ*被发现定位在有丝分裂期的动点上, 并且作为分子伴侣帮助稳定了Bub3蛋白, 同时也介导了Bub3复合物的动点定位[66-67]。

以上提到的蛋白对SAC蛋白的稳定性以及动点定位都有着直接的重要作用, 然而它们在此方面发挥功能的机制还不十分清楚, 对这些蛋白功能发挥的进一步研究无疑对更加深刻地了解SAC有着重要的意义。

3 SAC的信号强度

在有丝分裂期, 如果仅存在一个未被微管正确连接的染色体就能将细胞阻断好几个小时, 所以长期以来SAC的信号强度一直被认为是“开或关”的状态。然而近期的三篇文章共同证实SAC的强度类似可变电阻, 大小可变[68-70]。在所有染色体都排列好的细胞中, 利用激光切断微管造成少数移位的染色体, 虽然大多细胞都被长期阻断, 仍然有将近30%的细胞在一段时间的阻断后能够进入后期。在这些可进入后期的细胞中, 随着移位染色体数量的增加, 阻断的时间也相应延长, 并且细胞中Securin的降解速度也变慢[69], 说明SAC的活性并不是“开或关”模式, 而具有大小之分。SAC活性的强度与动点上Mad2的蛋白量直接相关, Mad2在动点上定位越多, APC/C被抑制的程度越高, 说明SAC的活性越强[68]。不仅如此, 动点定位的Mad2和胞质中Mad2的蛋白量以及细胞中CDC20蛋白水平与SAC的强度息息相关。在酵母中, 利用启动子强弱使Mad2的表达量降低50%, 导致SAC的活性也下降; 然而同时也降低CDC20蛋白水平, SAC的活性又得以恢复, 说明SAC活性的强弱主要体现在细胞中CDC20被MCC屏蔽的程度。与此观点一致的是, 将细胞中Mad1的表达量提升至300%~500%, 细胞中的Mad2将被全部招募到动点上, 导致SAC活性的大大下降[70]。

4 SAC的失活机制

当动点被微管捕获后, SAC将逐渐失活, 然而SAC失活的机制尚不十分明了。整个SAC失活过程可能包含了三个部分: 首先是从源头阻断MCC复合物的形成; 其次是对胞质中游离的MCC复合物进行去组装; 第三部分是对结合在APC/C上的MCC复合物进行去组装[71]。

动点–微管连接直接触发了SAC蛋白从动点上的解离, 从而阻断了MCC复合物的形成。一个被广泛接受的机制是, 马达蛋白Dynein将动点上的Mad1、Mad2、Mps1等SAC蛋白沿着微管运输到中心体上[6]。在这个过程中, Spindly和LIC1(dynein light intermediate chain 1)蛋白发挥着重要作用, 其中Spindly负责Dynein的动点定位, 并且在SAC失活时, Spindly本身也是通过Dynein的运输从动点上解离的[72-73]; 当细胞中LIC1的表达被降低时, SAC蛋白从动点上的解离大大受到影响, 原因是Mad2和Dynein之间的结合受到影响, 所以LIC1可能直接介导了Dynein和Mad2之间的结合[74]。同时, p31(comet)与Mad2之间的结合阻碍了O-Mad2向C-Mad2的转变, 从而抑制了MCC复合物的形成[75]。

对于未结合APC/C的游离MCC, p31(comet)和CDK激酶在其去组装过程中共同发挥着重要的作用。p31(comet)与MCC复合物中的C-Mad2结合解除CDC20与BubR1之间的相互作用, 并且这个过程依赖于ATP的水解[76]。如果突变CDC20上面CDK激酶的磷酸化位点, p31(comet)介导的MCC复合物去组装将受到显著的抑制, 说明CDK激酶介导的CDC20磷酸化在MCC复合物解离的过程中发挥重要的作用, 同时p31(comet)的存在促进了CDC20的磷酸化[77]。综合以上的研究结果, p31(comet)与

MCC复合物中的Mad2结合, 引发CDC20的构象改变, 促进CDK激酶对CDC20进行磷酸化, 破坏CDC20与BubR1的相互作用[76-78]。

对于结合在APC/C复合物上的MCC, 其去组装过程依赖于APC/C对MCC成员的多泛素化修饰, 从而破坏Mad2和BubR1与CDC20的结合[79]。其中, APC/C复合物的亚基APC15促进CDC20的多泛素化, 并且催化泛素化的CDC20从MCC复合物上解离[80-81]。解离的CDC20和BubR1将进入泛素依赖的蛋白降解途径。在此之前, 乙酰转移酶PCAF介导的乙酰化修饰阻止了BubR1的降解[82-84]。CUEDC2蛋白在SAC失活的过程中也发挥着重要的作用。CDK激酶通过介导CUEDC2蛋白的磷酸化, 促进CUEDC2与CDC20的结合, 从而调控Mad2从MCC复合物上解离, 达到调节SAC失活的目的[85]。

在SAC失活过程中, 磷酸酶PP1的动点定位也同样发挥着重要的作用[86-88], 而其中的机制还有待研究。一个可能的原因是PP1通过对KNL1上磷酸化的MELT序列进行去磷酸化, 从而去除Bub3的结合位点, 致使SAC蛋白完全从动点上解离[22]。值得关注的是, 同Bub1、BubR1/Mad3和Bub3一样, PP1的动点定位也是由KNL1部分介导的[88-89]。

5 SAC蛋白的其他功能

检验点蛋白除了在SAC信号发生中发挥功能外, 还对有丝分裂进程的其他方面有着重要的作用。BubR1在染色体排列以及动点–微管连接方面也发挥着重要作用[90-92]。磷酸酶PP2A促进了动点–微管的连接[93]。在最近的报道中, BubR1的630-720氨基酸区域被发现与PP2A的调节亚基B56直接相互作用, 从而招募PP2A的动点定位, 并且BubR1与B56之间的相互作用保证了染色体的正常排列[94-95]。Plk1激酶介导的磷酸化对BubR1在动点–微管连接方面的功能也有着重要的作用[96]。有趣的是, Plk1的磷酸化位点BubR1-T676位于B56的结合区域内, Plk1是否通过对T676位的磷酸化调控BubR1与B56-PP2A的相互作用还有待进一步的研究。马达蛋白CENP-E被证明是BubR1的激活子并能促进BubR1的自磷酸化[92]。BubR1和CENP-E-BubR1复合物的结构解析以及基于结构筛选的特异性小分子抑制剂将会为理解BubR1的细胞学功能提供坚实的基础。

与BubR1不同的是, Bub1的结构在进化上比较保守, 然而同样的是Bub1的激酶功能主要介导了染色体的排列而对SAC的作用不显著[97]。Bub1的一个重要的底物是组蛋白H2A。通过对着丝粒附近的H2A-T120磷酸化, 招募Shugoshin(Sgo)蛋白从而介导姐妹染色单体的连接[51,98-100]。由于Sgo与CPC复合物(chromosome passenger complex, 包括Incenp、Survivin、Borealin和Aurora B激酶)中的Borealin相互作用可促进Aurora B的着丝粒定位, 所以Bub1同时也通过Aurora B在纠正错误的微管连接方面发挥功能[101]。

利用Mps1的小分子抑制剂, Santaguida等[102]发现Mps1在动点–微管连接的纠错方面也发挥着重要的作用。然而同Bub1一样, 这方面的功能可能也是通过Aurora B激酶来实现的。因为Mps1被证实可以通过磷酸化Borealin增强Aurora B的激酶活性[103]。如前所述, Aurora B又促进Mps1的动点定位, 所以在体内Bub1、Mps1和Aurora B三个激酶之间可能相互促进, 形成一个激酶环路[52]。马达蛋白CENP-E在染色体排列以及动点–微管连接方面发挥着重要的作用, 其N-端的马达和C-端的尾部存在着分子内的自抑制。Mps1的另一个可能的重要功能是通过磷酸化CENP-E的尾部来解除其分子内的自抑制[104]。

6 结论

自从诺贝尔获得者Lee Hartwell在1989年证实了细胞周期检验点的存在和随后1991年SAC的发现, SAC的研究已经走过了二十多年的历程。伴随着生命科学研究手段的进步, SAC的组成因子与作用机制逐渐被揭开面纱。同时, 对于动点的结构与功能解析特别是动点与微管作用界面的新发现极大地促进了SAC的研究。多个关键的SAC激酶的动点定位的结构基础在过去3年内被揭示。同时, 对于SAC信号转导机制的体外重组实验体系的建立和MCC复合物结构生物学亦取得了重要的突破。我们期待在超高分辨率显微镜技术等新技术的帮助下, SAC的信号通路与分子机制在未来会被全面地揭示。

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