

## 二型态真菌形态调控研究现状与展望

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**摘要:** 真菌二型态(Dimorphism)是指某些真菌在外界环境因子的诱导下,其营养体可在酵母型(Yeast form)和菌丝型(Mycelium form)两种不同细胞形态间转化的能力。诱导二型态真菌形态转化的环境因子众多,不同环境因子诱导二型态转化的分子机制也不尽相同。二型态真菌形态转换常与致病性有关,在微生物发酵调控中也至关重要,研究真菌形态调控不仅有助于阐明二型态真菌的致病机理,还可丰富二型态真菌形态调控在发酵工业中的应用。本文从影响真菌二型态的外界环境因子、转化机制和真菌发酵形态控制等方面对二型态真菌形态调控研究现状及应用前景进行综述。

**关键词:** 二型态真菌, 形态转换, 环境因子, 形态调控, 分子机制

## Advances in dimorphism transition in fungi

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**Abstract:** Dimorphism refers to the peculiar capacity of several fungi to shift morphologically between yeast and mycelial forms in response to environmental factors. Various signaling pathways connect external stimuli and cell morphological changes. Although the dimorphism transition is usually related to pathogenicity, it also greatly affects cell behavior during fermentation. Investigating the morphogenetic shifts of those fungi not only builds the foundation for elucidating pathogenic mechanisms, but may also help identify novel regulation strategies for biotechnological

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applications. The present work focuses on the latest researches aimed at understanding the environmental factors and their underpinning molecular mechanisms, discusses potential biotechnological applications, and provides a comprehensive basis for further research.

**Keywords:** Dimorphism fungi, Morphological switch, Environmental factors, Morphology control, Molecular mechanism

真菌二型态(Dimorphism)又称二型性、二相性或二型现象,是指一些真菌在某些环境因子的影响下,使菌体形态在酵母型与菌丝型两种形态之间发生相互转变的能力<sup>[1-2]</sup>(图 1)。具有二型态能力的真菌很多,在子囊菌、担子菌和接合菌中均存在,其中尤以子囊菌居多。目前研究报道较多的典型二型态真菌主要有:玉米黑粉菌(*Ustilago maydis*)、白假丝酵母(*Candida albicans*)、酿酒酵母(*Saccharomyces cerevisiae*)、解脂酵母(*Yarrowia lipolytica*)和银耳(*Tremella fuciformis*)等(表 1)。研究表明,大多数二型态真菌的形态转换与其营养摄取和致病性密切相关<sup>[3]</sup>。例如酿酒酵母在碳源缺乏的固态琼脂培养基上以侵入性强的酵母型出现,并能在半固体培养基上形成生物膜;而在氮饥饿时,则能转换为假菌丝型<sup>[13]</sup>。刘娟等<sup>[23]</sup>发现在不同碳氮比条件下二型态银耳节孢子会在酵母型细胞和菌丝型之间进行转换。相对非致病真菌而言,二型态致病真菌的形态转换与入侵及粘附细胞、分泌胞外蛋白酶等致病过程有关。例如白假丝酵母菌的菌丝型细胞对入侵寄主是必需的,不能形成菌丝的突变体,其致病能力降低或消失<sup>[25]</sup>;申克氏孢子丝

菌(*Sporothrix schenckii*)的酵母型能引起皮肤、皮下组织、勃膜和局部淋巴系统甚至全身多系统的损害,而菌丝型则无致病性<sup>[10]</sup>。因此,研究致病二型态真菌的生理特性和诱发致病的环境因素,将有助于控制致病二型态真菌的形态,最终控制其致病性。

此外,菌体形态调控作为微生物发酵调控的重要内容,对于提高真菌在工业发酵中的生产水平具有重要作用,受到高度重视。以菌丝型生长的真菌会形成分散菌丝体(Dispersed mycelium)、球状菌丝体(Pellet)和团簇状菌丝体(Clump or Flock)等 3 种集结形态,不论哪种形态,都会对发酵液的流体学性质、营养物质摄取和氧气传递带来影响<sup>[26-28]</sup>。随着细胞菌丝的增多,真菌细胞间更容易发生结团现象,从而使发酵液的粘度不断增加,进一步阻碍细胞对营养物质的摄取和氧气的传递,最终影响菌体的生长和产物的生成<sup>[29]</sup>。为此,研究人员根据真菌生长的特性,通过优化培养基的成分及组成来控制丝状真菌形态。如 Christiansen 等<sup>[30]</sup>研究了不同葡萄糖浓度下黑曲霉(*Aspergillus niger*)菌丝体的生长情况,发现在不同糖浓度下,菌丝

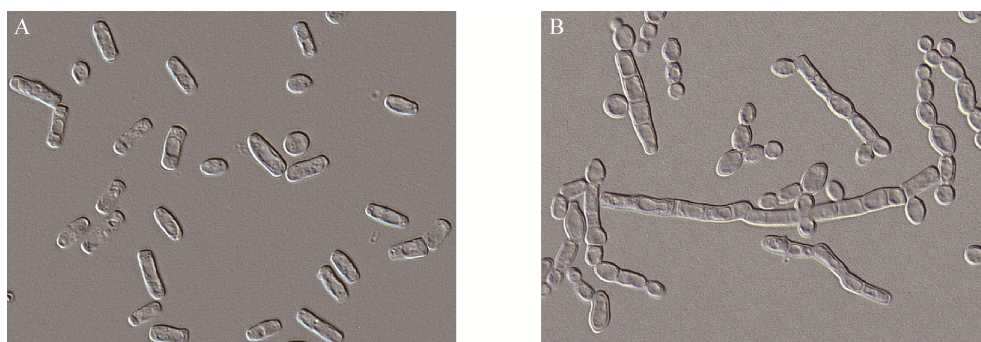


图 1 二型态真菌皮肤丝孢酵母的两种形态(1 000×)

Figure 1 Two morphologies of dimorphic fungi *Trichosporon cutaneum* (1 000×)

注: A: 酵母型; B: 菌丝型。

Note: A: Yeast form; B: Mycelium form.

表 1 常见二型态真菌种类  
Table 1 Summary of dimorphic fungal diversity

真菌门 Eumycota	种名 Species	致病形态 Pathogenic form	参考文献 References
子囊菌门 Ascomycota	白假丝酵母 <i>Candida albicans</i>	菌丝型 Filament	[3]
	杜氏假丝酵母 <i>Candida dubliniensis</i>	菌丝型 Filament	[4]
	热带假丝酵母 <i>Candida tropicalis</i>	菌丝型 Filament	[5]
	近平滑假丝酵母 <i>Candida parapsilosis</i>	菌丝型 Filament	[6]
	葡萄牙假丝酵母 <i>Candida lusitanae</i>	菌丝型 Filament	[7]
	巴西副球孢子菌 <i>Paracoccidioides brasiliensis</i>	酵母型 Yeast	[8]
	荚膜组织胞浆菌 <i>Histoplasma capsulatum</i>	酵母型 Yeast	[9]
	粗球孢子菌 <i>Coccidioides immitis</i>	酵母型 Yeast	[9]
	申克孢子丝菌 <i>Sporothrix schenckii</i>	酵母型 Yeast	[10]
	皮炎芽生菌 <i>Blastomyces dermatitidis</i>	酵母型 Yeast	[9]
	发酵毕赤酵母 <i>Pichia fermentans</i>	—	[1]
	日本裂殖酵母 <i>Schizosaccharomyces japonicus</i>	—	[11]
	酿酒酵母 <i>Saccharomyces cerevisiae</i>	—	[12]
	非洲栗酒酵母 <i>Saccharomyces pombe</i>	—	[13]
	解脂假丝酵母 <i>Yarrowia lipolitica</i>	—	[14]
	马内菲青霉菌 <i>Penicillium marneffei</i>	酵母型 Yeast	[9]
	榆树枯萎病菌 <i>Ceratocystis ulmi</i> , <i>Ophiostoma novo-ulmi</i> , <i>Ophiostoma ulmi</i> , <i>Ophiostoma himal-ulmi</i>	菌丝型 Filament	[15]
	长喙壳状表皮癣菌 <i>Ophiostoma floccosum</i>	菌丝型 Filament	[16]
	玫烟色拟青霉菌 <i>Isaria fumosorosea</i>	菌丝型 Filament	[17]
	烟曲霉 <i>Aspergillus fumigatus</i>	菌丝型 Filament	[18]
	出芽短梗霉菌 <i>Aureobasidium pullulans</i>	—	[19]
	串珠镰刀菌 <i>Fusarium moniliforme</i>	菌丝型 Filament	[20]
	稻瘟病菌 <i>Magnaporthe grisea</i>	菌丝型 Filament	[18]
担子菌门 Basidiomycota	新型隐球菌 <i>Cryptococcus neoformans</i>	酵母型 Yeast	[9]
	格特隐球菌 <i>Cryptococcus gattii</i>	酵母型 Yeast	[9]
	玉米黑粉菌 <i>Ustilago maydis</i>	菌丝型 Filament	[21]
	茭白黑粉菌 <i>Ustilago esculenta</i>	菌丝型 Filament	[22]
	银耳 <i>Tremella fusiformis</i>	—	[23]
	糖秕马拉色菌 <i>Malassezia furfur</i>	酵母型 Yeast	[24]
接合菌门 Zygomycota	卷枝毛霉 <i>Mucor circinelloides</i>	菌丝型 Filament	[9]

注：—：未提及。

Note: —: Non-described.

体的分枝情况会有所区别。桑美纳等<sup>[31]</sup>采用硫酸-豆油偶联型流加策略,使顶头孢霉(*Cephalosporins acremonium*)细胞在发酵中期正常分化为高度膨胀菌丝体,减少菌丝结球率,实现了头孢菌素 C 的高产。Liu 等<sup>[32]</sup>全面考察了接种孢子、葡萄糖、尿

素、磷酸盐、金属离子的浓度和发酵体系 pH 及添加微粒子对米根霉(*Rhizopus oryzae*)生长形态的影响及其变化规律。

就二型态真菌而言,虽然人们对其形态转换已有较多研究,但大都集中在探索致病真菌的感染机

理方面,而探讨如何将二型态真菌的形态调控应用于工业化发酵生产上的研究相当缺乏。因此探索二型态真菌细胞形态调控的环境因子,实现其在发酵过程中形态的有利转换,不但可以促进菌体的生长和产物的合成,实现发酵后期产物的高效分离,而且对于提高其发酵效率 and 生产水平都具有重要意义。本文就近年来影响真菌二型态的外界环境因子、转化机制和真菌发酵形态控制等方面的研究进展进行综述,以期为阐明二型态真菌的转化机理,丰富和提升真菌形态调控的内容和认识水平提供参考。

## 1 调控二型态真菌形态转换的环境因子

环境因子是二型态真菌形态转换过程中的最初启动因子。对于不同的二型态真菌,影响其形态转换的环境因子也不尽相同,因此探索环境触发因子是研究二型态真菌形态转换的首要任务。目前,关于触发二型态真菌形态转换的环境因子主要有以下几类。

### 1.1 营养因子

营养因子主要是指菌体生长过程中所必需的一些营养成分,如氮源、氨基酸、碳源等,其中氮源是最重要的因子之一。白假丝酵母菌在氮限制情况下,铵离子透性酶 Mep2 能诱导其细胞由酵母型向菌丝型转变<sup>[33]</sup>。Lee 等发现新型隐球菌在正常培养条件下细胞呈酵母型,而当氮源限制时则以假丝状生长<sup>[34]</sup>。此外,酿酒酵母菌在氮饥饿时细胞也

能够从酵母型转变为假菌丝型<sup>[35]</sup>;而解脂假丝酵母菌在氮源限制条件下,其细胞呈酵母状生长,菌丝型受抑制<sup>[16]</sup>。发酵毕赤酵母(*Pichia fermentans*)的细胞形态对氮源浓度高度依赖,在培养基中尿素和磷酸氢二铵的浓度为毫摩尔级时主要以酵母型生长为主,而在微摩尔级则呈假丝状<sup>[1]</sup>。本课题组也发现,氮源浓度对于皮状丝孢酵母(*Trichosporon cutaneum*)的形态转换至关重要,正常情况下,皮状丝孢酵母在发酵过程中是以丝状生长为主(图 2A),而在氮源限制的情况下,其形态能发生变化,由菌丝型为主变为酵母型为主(图 2B),增加氮源的供应,细胞又会转为菌丝型生长为主(图 2C)<sup>[36]</sup>。

此外,研究人员还发现氮源种类,尤其是氨基酸对二型态真菌的菌丝形态变化也有重要影响。如发酵毕赤酵母在含可同化氮源(如酵母提取物、蛋白胨、尿素、磷酸氢二铵等)的培养基中主要呈酵母型;而在含硫酸铵、甲硫氨酸、缬氨酸和苯丙氨酸等作为氮源的培养基中则转化形成假丝<sup>[1]</sup>。Nickerson 最早发现白假丝酵母菌在淀粉培养基上可以产生菌丝和厚膜孢子<sup>[37]</sup>,在添加了半胱氨酸后就只产生酵母细胞,而在含有 8 种氨基酸(丙氨酸、亮氨酸、赖氨酸、甲硫氨酸、脯氨酸、苯丙氨酸、鸟氨酸、苏氨酸)的 Lee 培养基中可以形成菌丝<sup>[38]</sup>。de Lima 等也研究发现氨基酸可使近平滑假丝酵母(*Candida parapsilosis*)的菌落形态和菌丝形态都发生明显变化,特别是瓜氨酸能促使其细胞形成假丝状<sup>[10]</sup>。

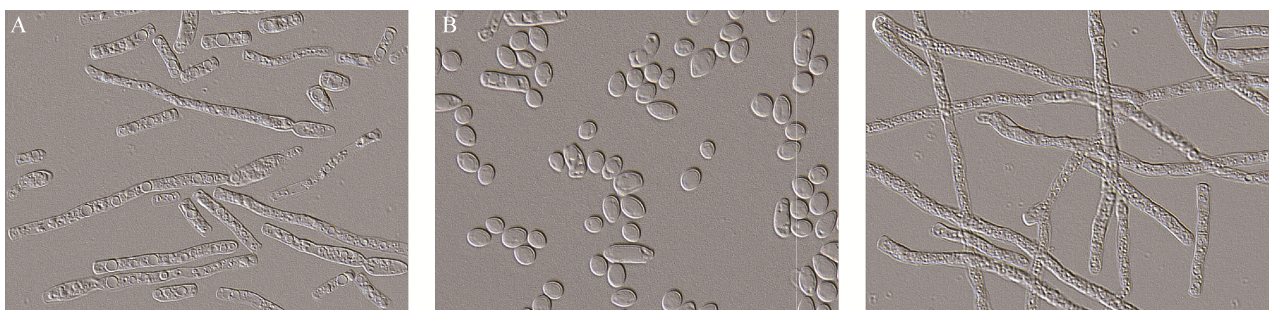


图 2 发酵过程中皮状丝孢酵母的两种形态(1 000×)

Figure 2 Two morphologies of *Trichosporon cutaneum* B3 (1 000×)

注: A: 正常发酵条件; B: 氮源限制; C: 氮源充足。

Note: A: Normol condition; B: Nitrogen limitation; C: Nitrogen abundance.



碳源种类也会影响真菌二型态的转换,如乳糖、麦芽糖、半乳糖和 N-乙酰葡萄糖胺等能促进白假丝酵母菌的菌丝型发育,但乙醇、甘油和  $\alpha$ -脱氧葡萄糖等会抑制其菌丝型的发育<sup>[39-40]</sup>。此外,研究者也发现在卷枝毛霉(*Mucor circinelloides*)的形态发育中,酵母型的形成需要己糖存在<sup>[41]</sup>。解脂假丝酵母菌在含蓖麻油或橄榄油等疏水性碳源的培养基中,菌体呈单细胞酵母型生长,而在以葡萄糖或 N-乙酰葡萄糖胺等作为亲水性碳源进行培养时,菌体则转换为菌丝型<sup>[42]</sup>。葡萄糖浓度同样对真菌形态转换有显著影响,如酿酒酵母在葡萄糖限制条件下呈菌丝型生长<sup>[43-44]</sup>;Buu 等研究发现,低葡萄糖浓度(约 0.1%)能刺激白假丝酵母菌菌丝体的形成<sup>[45]</sup>。

## 1.2 化学因子

有些二型态真菌在其形态转换时需添加一些化学物质进行诱导。如脂类物质能促进玉米黑粉菌菌丝型生长,而高级饱和脂肪酸、高级不饱和脂肪酸、厚朴酚以及肉桂醛等对白假丝酵母菌菌丝型的发育有抑制作用<sup>[46-48]</sup>,氧化脂类、水杨酸等会诱导荷兰榆树病原真菌(*Ophiostoma ulmi*)呈菌丝型生长<sup>[49]</sup>。尽管荚膜组织胞浆菌(*Histoplasma capsulatum*)的二型态受温度控制,但控制其酵母型生长的基因仅能在高温且同时存在巯基的培养条件下才能表达<sup>[50]</sup>。血清能很好地诱导解脂酵母、日本裂殖酵母(*Schizosaccharomyces japonicus*)等菌丝的形成<sup>[51]</sup>。

## 1.3 物理因子

物理因子主要包括温度、pH 以及气体环境等。迄今为止,已经发现很多二型态真菌的形态转换与温度密切相关。例如巴西副球孢子菌(*Paracoccidioides brasiliensis*)是一种典型的依赖于温度的二型态真菌,在 20 °C 时呈菌丝型,37 °C 时呈酵母型<sup>[12]</sup>,马尔尼菲青霉菌(*Penicillium marneffei*)也是一个对温度高度依赖的二型态真菌<sup>[52]</sup>。Schwartz 等<sup>[13]</sup>曾报道粗球孢子菌(*Coccidioides immitis*)、荚膜组织胞浆菌(*Histoplasma capsulatum*)、申克孢子丝菌和皮

炎芽生菌(*Blastomyces dermatitidis*)均在 20–25 °C 时呈菌丝型,而当温度升高至 37 °C 时则转化为具有致病性的酵母型;但白假丝酵母则相反,其在 25 °C 为酵母型,在 37 °C 为菌丝型。在正常条件下(pH 7.0),玉米黑粉菌以酵母型存在,而在酸性条件下(pH 3.0)则以菌丝型生长<sup>[19]</sup>。在低溶氧条件下(DO<3%),玫瑰色拟青霉菌(*Isaria fumosorosea*)以菌丝型生长为主,而高溶氧条件下(DO>20%)则有利于酵母型细胞积累<sup>[22]</sup>;而卷枝毛霉(*Mucor circinelloides*)则相反,其在有氧条件下呈菌丝型生长,在厌氧条件或高 CO<sub>2</sub> 浓度则转化为酵母型生长<sup>[53]</sup>。对于解脂假丝酵母,半厌氧状态更有利于其呈菌丝型生长<sup>[54]</sup>。高 CO<sub>2</sub> 浓度能有效促进格特隐球菌(*Cryptococcus gattii*)形成大量单核菌丝体及孢子<sup>[55]</sup>。在低于 37 °C 情况下,高 CO<sub>2</sub> 浓度也可以促进白假丝酵母菌形成菌丝型生长<sup>[56]</sup>。

## 1.4 其他环境因子

除了上述三类环境因子外,还存在一些其他环境因子也可诱导真菌二型态。榆枯萎病菌(*Ceratocystis ulmi*)在葡萄糖-脯氨酸-盐的培养基中,当接种量大于 10<sup>6</sup> CFU/mL 时,其细胞发育成酵母型;相反,当接种量小于 10<sup>6</sup> CFU/mL 时,细胞则成菌丝型,而其他环境因子对其没有影响<sup>[17]</sup>。长喙壳状表皮癣菌(*Ophiostoma floccosum*)也存在相同的现象,在以 L-脯氨酸作为氮源的培养基中,当接种量为 10<sup>7</sup> CFU/mL 时能极大地促进其呈酵母型生长,而当接种量为 5×10<sup>5</sup> CFU/mL 时则促使其菌丝型生长<sup>[18]</sup>。接种量对真菌形态的这种影响可通过群体效应(Quorum sensing)进行解释,即在某些特定条件下,菌体自身会分泌一些群体感应分子,这些群体感应分子的浓度会随着菌体密度的增加而升高,当超过一定的阈值后就会调节菌体的一些生理生化特性发生相应的改变<sup>[57]</sup>。通常,一些高级醇可作为群体感应分子来调节二型态真菌的形态转换,如法尼醇能抑制白色念珠菌的出芽及生物膜的形成<sup>[58]</sup>,而甲硫醇、正丁醇、异丁醇和异丙醇能诱导发酵毕赤酵母呈假菌丝型生长<sup>[1]</sup>,在酿酒酵母中,

异戊基乙醇能通过由 Swel1 蛋白激活的 Cdc28 磷酸化作用而诱导菌丝的形成<sup>[59]</sup>。2-甲基-1-丁醇、4-羟基苯乙酸和 3-甲基戊酸作为荷兰榆树病菌的群体感应分子,能抑制其至少 50%的菌体形成芽管<sup>[60]</sup>。

## 2 二型态真菌形态转换的机制

### 2.1 信号转导途径

真菌二型态转换的机制是目前研究的热点,迄今已有大量报道,研究内容主要集中在真菌二型态的信号转导途径和基因水平调控等方面<sup>[3,16]</sup>。关于二型态真菌的信号转导途径,研究人员已经发现了真菌在形态转换过程中涉及到内部多条信号转导

途径,主要包括 MAPK 途径(Mitogen-activated protein kinase pathway)、cAMP-PKA 途径(cAMP-protein kinase A pathway)、TOR (Target of rapamycin pathway)途径、Rim101 途径和  $\text{Ca}^{2+}$ /Calcineurin 途径等<sup>[8,61-64]</sup>。

MAPK 途径是调控二型态真菌形态转换过程的重要途径之一(图 3),在酿酒酵母中,Ste11、Ste7 和 Kss1 依次作为 MAPKKK (Mitogen-Activated Protein Kinase Kinase Kinase)、MAPKK (Mitogen-Activated Protein Kinase Kinase)和 MAPK (Mitogen-Activated Protein Kinase)将接收到的外界信号逐级

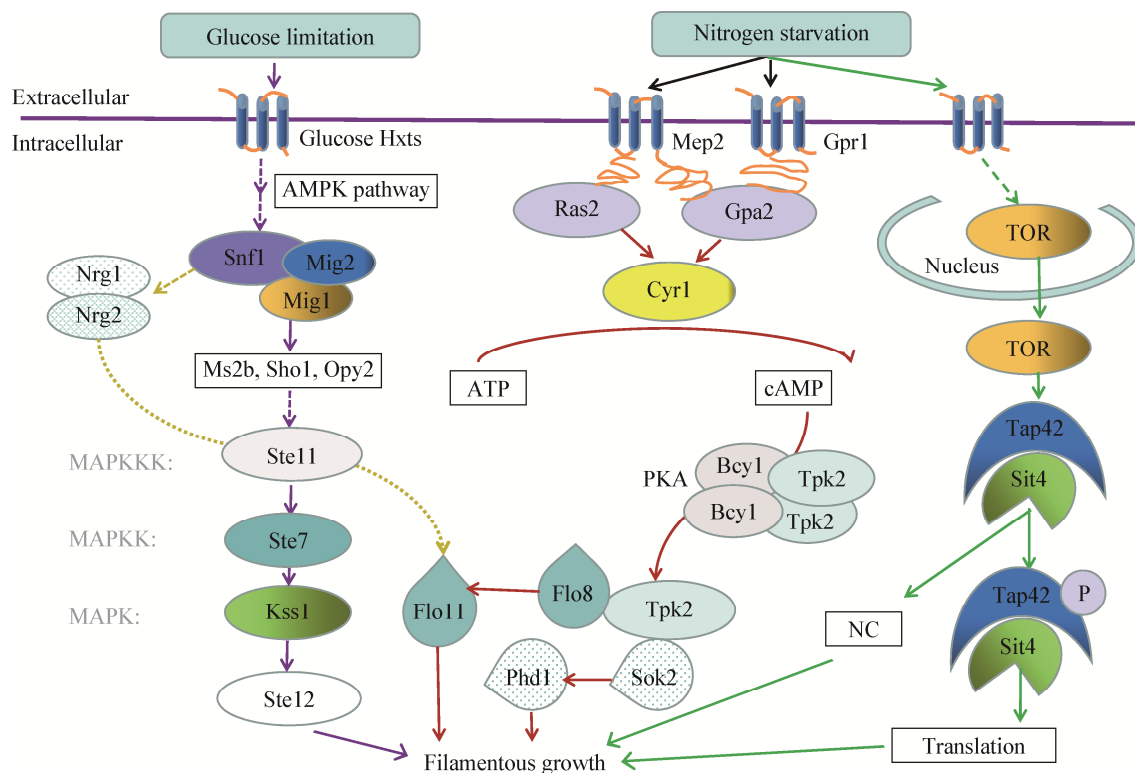


图 3 酿酒酵母菌丝形成的主要信号通路

Figure 3 Multiple signaling pathways that control filamentous growth in *S. cerevisiae*

注: Snf1 蛋白激酶对于响应葡萄糖限制的许多细胞反应都十分重要。AMPK 途径中的 Snf1 与 Nrg1 和 Nrg2 以及 Mig1 和 Mig2 都是促进菌丝生长所必需的。小 G 蛋白 Ras2 是 MAPK 通路与 cAMP 通路中共同的上游蛋白,能够调节菌丝发育。Ras2p 或 Gpa2p 可活化腺苷酸环化酶(Cyr1p),生成的 cAMP 进而诱导特定基因的表达促使菌丝形成。Tor 蛋白激酶在营养传感信号转导通路中发挥作用,当其与雷帕霉素结合后活性受到抑制。TOR 信号级联反应途径主要通过氮阻遏(NCR)和 Tap42-Sit4 磷酸酶复合物调控菌丝的生长。

Note: The Snf1, protein kinase that is important for many cellular responses to glucose limitation. The AMPK Snf1, Nrg1, Nrg2, Mig1 and Mig2, were required for filamentous growth in response to glucose limitation. The small G protein Ras2, an important regulator of hyphal development and likely functions upstream of both MAPK pathway and cAMP pathway. Adenylate cyclase (Cyr1p) can be activated by Ras2p or Gpa2p, inducing the expression of specific genes to bring about filamentous growth. Rapamycin binds and inhibits the Tor protein kinases, which function in a nutrient-sensing signal transduction pathway. TOR signaling cascade controls filamentous growth via nitrogen catabolite repression (NCR) and the Tap42-Sit4 phosphatase complex.

放大<sup>[65]</sup>。在葡萄糖限制条件下, AMPK (Adenosine 5'-monophosphate (AMP)- activated protein kinase) 途径中的 Snf1 蛋白激酶可作用于 Mig1 和 Mig2 转录抑制因子, 然后由 Mig1/2 联合 MAPK 途径共同调节其菌丝体形成, 同时 AMPK 途径中的 Snf1 也可通过 Snf1-Nrg 途径诱导 *Flo11* 基因的表达, 促进菌丝形成; 而酿酒酵母在氮饥饿等营养条件的刺激下, 膜受体蛋白 Gpr1 和 Mep2 将信号传递至小 G 蛋白 Ras2 和 Gpa2, 从而激活腺苷酸环化酶生成 cAMP, cAMP 能激活 PKA, 从而促进菌丝型的生长<sup>[66-68]</sup>(图 3)。氮饥饿还可诱发酿酒酵母启动 TOR 途径调节其菌体形态转换, TOR 激酶是酿酒酵母感知外界营养条件的一个重要激酶, 在氮源缺乏或雷帕霉素处理后, TOR 蛋白会从细胞核转向细胞质, 然后作用于其下游元件 Tap42 和 Sit4, 进而促发氮源阻遏效应引起菌体形态改变, 或由磷酸化的 Tap42-Sit4 复合体促进相关基因的转录与表达, 从而使菌体呈菌丝型生长<sup>[69]</sup>(图 3)。

Rim101 途径对于白假丝酵母、酿酒酵母、新型隐球菌等二型态真菌响应环境 pH 而引起的形态分化具有重要作用<sup>[70-72]</sup>。在碱性条件下, 白假丝酵母中受 pH 调节的 *phr1* 基因高效表达, 编码形成膜上糖苷酶, 膜受体蛋白 Rim9 及 Rim21 接收信号并将其传至 Rim8 与 Rim20 蛋白, Rim20 与 Rim101 的 C 端结合, 在 Rim13 的酶切作用下, 全长的 Rim101 水解形成较短的 Rim101 而具有活性, 进而诱导基因 *efg1* 表达形成菌丝(图 4)<sup>[73]</sup>。*phr2* 为细胞膜上糖苷酶的另一个编码基因, 其只有在酸性条件下表达, *phr2* 基因缺陷型突变菌株在 pH 低于 5.5 时呈菌丝型生长, 说明 *phr2* 基因能抑制菌丝体形成<sup>[74-75]</sup>。

$\text{Ca}^{2+}$ /Calcineurin 途径则主要参与卷枝毛霉、烟曲霉、稻瘟病菌(*Magnaporthe grisea*)和念珠菌属中一些二型态真菌的形态转换<sup>[8-9]</sup>。如热带假丝酵母(*Candida tropicalis*)在血清等外界环境的作用下, 细胞外的  $\text{Ca}^{2+}$  进入细胞内并与钙调蛋白结合形成  $\text{Ca}^{2+}$ /CaM 复合体。无活性的钙调磷酸酶与

$\text{Ca}^{2+}$ /CaM 复合体结合而具有活性, 活化的钙调磷酸酶进一步作用于 Crz1 转录因子, 使其去磷酸化, 去磷酸化的 Crz1 进入细胞核调节相关菌丝形成基因的表达(图 5)<sup>[76]</sup>。钙调磷酸酶在卷枝毛霉的二型态转换过程中也起至关重要的作用, 它能诱导卷枝毛霉由酵母型向菌丝型转变并抑制其细胞内 PKA 激酶的活性。卷枝毛霉中的钙调磷酸酶含 3 个催化亚基 A (CnaA、CnaB、CnaC) 以及 1 个调节亚基 B (CnbR), CnbR 对维持钙调磷酸酶的活性十分重要。敲除编码 CnbR 基因的突变株或用钙调磷酸酶抑制剂 FKBP12-FK506 或 CypA-CsA 处理后的菌株, PKA 的活性明显升高, 菌体呈酵母型生长<sup>[77-78]</sup>。

## 2.2 基因表达差异

由于二型态真菌的信号转导途径中蛋白酶起到关键的作用, 信号分子和调节蛋白从本质上都是受到基因表达的调控, 因此研究人员更乐于在基因

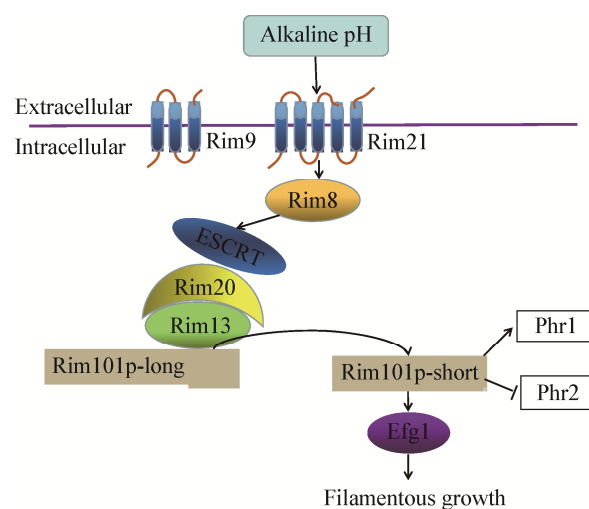


图 4 碱性 pH 条件下, Rim101 途径参与白假丝酵母菌丝形成

### Figure 4 Rim101 signaling pathway that controls filamentous growth in *C. albicans*

注: Rim9 和 Rim21 都是膜受体蛋白。全长 Rim101 可以被 Rim13 蛋白酶水解。水解后的短链 Rim101p 具有活性, 对于应答碱性环境的各种反应至关重要, 包括碱性诱导基因的活化、碱性抑制基因的抑制以及菌丝的形成。

Note: Rim9 and Rim21, plasma membrane receptor proteins. Full-length Rim101 can be processed by Rim13, a calpain-like protease. Processed Rim101p-short is required for the alkaline response, which includes activation of alkaline-induced genes, repression of alkaline-repressed genes, and filamentation.

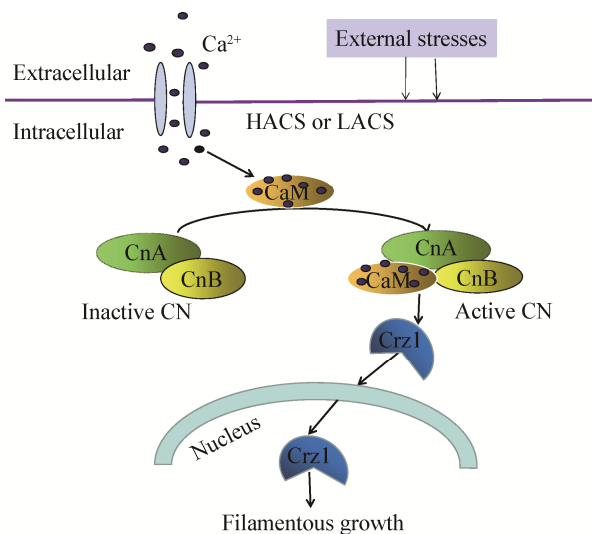


图 5  $\text{Ca}^{2+}$ /Calcineurin 途径参与的热带假丝酵母菌丝形成

Figure 5  $\text{Ca}^{2+}$ /Calcineurin signaling pathway that controls filamentous growth in *Candida tropicalis*

注: 钙调神经磷酸酶(CN)由催化亚基 A (Cna)和调节亚基 B (Cnb)组成, 能被  $\text{Ca}^{2+}$ /钙调蛋白复合物激活. 活化的钙调神经磷酸酶(CN)会促使转录因子 CRZ1 去磷酸化, 进而调节菌丝生长.

Note: Calcineurin (CN), consisting of the catalytic A (Cna) and regulatory B (Cnb) subunits, is activated by  $\text{Ca}^{2+}$ /Calcineurin complex. The transcription factor Crz1, dephosphorylated by activated CN then to regulate filamentous growth.

水平上对触发和调控二型态真菌发生形态转换的分子机制进行研究, 并希望寻找到与二型态转化有关的基因. 利用基因表达差异分析方法, 可以了解二型态真菌细胞在不同生理条件下或在不同生长发育阶段的基因表达差异, 从而为阐明二型态真菌的形态转换分子机制提供重要信息. 目前研究基因差异表达的主要技术有 mRNA 差异显示(mRNA differential display, DD)<sup>[79]</sup>、转录组测序技术(RNA sequencing, RNAseq)<sup>[80]</sup>、基因表达系列分析(Serial analysis of gene expression, SAGE)<sup>[81]</sup>、代表性差异分析(Representational difference analysis, RDA)<sup>[82]</sup>、抑制消减杂交法(Suppression subtractive hybridization, SSH)<sup>[83]</sup>、交互扣除 RNA 差别显示技术(Reciprocal subtraction differential RNA display, RSDD)<sup>[84]</sup>和 DNA 微列阵分析(DNA microarray analysis)<sup>[16]</sup>等. 如 Nigg 等利用 RNAseq 技术分别对酵母型和菌丝

型的荷兰榆树病菌进行测序, 发现两种形态下的菌体有将近 12%的基因表达存在差异<sup>[85]</sup>; Marques 等<sup>[86]</sup>利用 SSH 技术发现 *ags1* 和 *tsa1* 基因在巴西副球孢子菌的酵母型中有很高的表达, 在玉米黑粉菌的 cAMP 信号转导途径中, 利用 SSH 技术发现编码醛酮还原酶的 *uor1* 基因起到重要作用<sup>[87]</sup>, 而包含 3 个 RNA 识别元件和甘氨酸富集区的蛋白编码基因 *UmRrm75* 决定了其菌丝发育<sup>[88]</sup>; Liu 等<sup>[89]</sup>利用 SSH 技术也发现 6 个可能与马尔尼菲青霉菌的形态转换有关的基因; Chacko 等<sup>[90]</sup>利用正向遗传筛选法(Forward genetic screen)发现 *RZE1* 对隐球酵母的形态转换十分重要, *RZE1* 为长链非编码 RNA, 它能调节 *znf2* 的转录与输出, 而 *znf2* 是控制其形态变化的关键.

### 3 应用与展望

现有研究结果表明, 影响真菌二型态转换的环境因素各式各样, 不同的环境因子对同一二型态真菌形态转换的作用存在差异, 而且相同的环境因子对不同真菌的作用效果也不尽相同, 甚至截然相反. 各二型态真菌对外界环境刺激的应答的实质是细胞内各种酶、信号分子以及基因相互作用的结果. 迄今为止, 在影响真菌二型态转化的环境因子和分子机制等方面已开展了一些卓有成效的工作, 为其今后在生产、生活中的应用奠定基础.

#### 3.1 抗真菌药物研发

二型态真菌的形态与其致病性密切相关, 因此研究者希望通过控制真菌形态来改变其毒性. Guirao-Abad 等<sup>[91]</sup>研究发现抗真菌药物两性霉素 B 能通过抑制白假丝酵母芽管生长而发挥其抗真菌作用, 抗真菌药物可能作用于真菌形态转换途径中的某些酶或者基因. 这为今后研究提供了新的思路, 例如: 怎样根据二型态真菌的形态转换与其致病性的关系来开发新的抗真菌药物, 二型态真菌形态转换信号途径中的信号受体能否作为药物作用的靶位点从而达到抗真菌的目的, 不同细胞形态基因表达的差异是否同致病性有关等.



### 3.2 真菌发酵形态控制

除了研究菌体形态与致病性的联系外,探究菌体发酵形态与其代谢产物之间的关系也备受关注。Braga 等运用定量图像分析技术实时监测解脂假丝酵母菌在不同培养条件下的菌体形态以及不同形态菌体代谢产物如  $\gamma$ -癸内酯、胞内脂质等之间的差异<sup>[42]</sup>。Driouch 等<sup>[92]</sup>与 Wucherpennig 等<sup>[93]</sup>还分别于 2010 和 2011 年提出了形态代谢工程学(Metabolic engineering of morphology)的概念,即利用分子生物学原理与图像分析技术分析形态与物质、能量代谢之间的关系,通过分子生物学技术合理改造菌体形态,进而完成对形态与物质、能量代谢最优调控的应用性技术<sup>[92-94]</sup>。迄今为止,在青霉、曲霉等发酵过程中,将形态学结构信息与宏观生物量、底物消耗、产物合成速率相关联的形态结构动力学已是一种重要研究手段<sup>[26-31]</sup>。因此,开展二态真菌发酵形态调控,实现其在发酵过程中形态的有利转换,不但可以提高发酵产量,实现发酵后期产物的高效分离,还可丰富和提升真菌形态调控的内容和认识水平,这都将为现代发酵工业提供参考。

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