

芥菜开花负调因子SVP及FLC同源互作域筛选和作用强度分析

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Evaluation of Acting Domain and Strength Mediating the Protein Self-interactions of SVP and FLC in *Brassica juncea*

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摘要 为探明芥菜开花负调因子SVP、FLC自身聚合的分子机制及其蛋白作用模式,利用酵母双杂交体系,分别对SVP、FLC蛋白自身聚合及其作用强度进行研究。结果表明:酵母菌Y187转化子Y187-pGADT7SVP和Y187-pGADT7SVP2~5均能与酵母菌Y2HGold转化子Y2HGold-pGBKT7SVP融合,并可在选择性固体培养基QDO/X/A上长出蓝色菌落,而Y187-pGADT7SVP1×Y2HGold-pGBKT7SVP不能在QDO/X/A生长。说明SVP蛋白能自身聚合,且与截短体SVP2~5同源结合,SVP蛋白自身聚合需要核心作用域K域参与。尽管MI域不能单独介导SVP自身聚合,但它的存在却能使SVP自身聚合作用增强,C域有可能会削弱该作用。同时,Y2HGold-pGBKT7FLC和Y2HGold-pGBKT7FLC2~5也能与Y187-pGADT7FLC融合,同时激活报告基因AUR1-C、HIS3、ADE2、MEL1,FLC能与截短体FLC2~5同源互作。K域是FLC蛋白自身聚合必须的,I域会增强这一作用。SVP和FLC的核心作用域K域均由K1、K2和K3亚域组成,形成3个经典的α螺旋,K域有9个高度保守的氨基酸位点及蛋白互作的结构模体(亮氨酸拉链)。

关键词: 芥菜 截短体 SVP FLC 酵母双杂交

Abstract: For further study on the molecular mechanism and interaction model of SVP and FLC protein homologous dimerization in flowering control in *Brassica juncea* Coss. (mustard), the selfinteractions of SVP and FLC were detected by the yeast two-hybrid system. The yeast stains of pGADT7SVP or pGADT7SVP2-5 could mate with pGBKT7SVP, which grew on selective agar plates QDO/X/A (SD/-Ade/-His/-Leu/-Trp/X-α-Gal/AbA) with blue stains. However, Y187-pGADT7SVP1 and Y2HGold-pGBKT7SVP could not mate into zygote diploids to grow on selective plates DO/X/A. The results showed that SVP or SVP2-5 truncated forms could act with SVP itself to combine and form homodimers. K domain of SVP was the key amino acid region to independently mediate and determine the homologous dimerization. MI-domain of SVP alone could not induce the self-interactions of SVP, but enhance the strength of homologous interactions. However, C-domain of SVP could weaken the protein self-interaction strength. The yeast stains of pGBKT7FLC2-5 and pGADT7FLC could mate into zygotes and grew on selective agar plates QDO/X/A with blue stains. The DNA-BD and AD were brought into proximity to activate transcription of four independent reporter genes (AUR1-C, HIS3, ADE2, MEL1). FLC2-5 truncated forms and FLC protein could act with each other to form homodimers. It also indicated that K domain of FLC may play an important role in mediating the FLC homodimers. However, I-domain of FLC could strengthen the protein self-interactions. Alignment analysis of K domain sequence showed that K domain was consisted of three subdomains (K1, K2 and K3) and formed three α helixes. Nine high conservative amino acids existed in K domain. Leucine zippers, protein interaction motifs, lied in K domain.

Keywords: *Brassica juncea*, truncated forms, SVP, FLC, yeast two-hybrid system

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