IDENTIFICATION OF GENES INVOLVED IN THE SEGREGATION OF YEAST 2 MICRON PLASMID

ANNIE ALBERT DEMIN

UMS
UNIVERSITI MALAYSIA SABAH

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ABSTRACT

IDENTIFICATION OF GENES INVOLVED IN THE SEGREGATION OF YEAST 2 MICRON PLASMID

The 2 µ plasmid of *Saccharomyces cerevisiae* is a circular, stable, high-copy-number, extrachromosomal "selfish" DNA element without any selective advantage to its host. In addition to the plasmid-encoded stability system, consisting of the Rep1 and Rep2 proteins and the *STB* DNA, chromosome-encoded proteins that affect the fidelity of chromosome segregation were also found to affect plasmid segregation in a similar way. Seven genes that were previously reported to be involved in chromosome stability were investigated by gene disruption. Phenotypes produced by gene disruption may provide important clues for the gene function. Disruption fragments for *HOS1*, *RME1*, *HHT2*, *RPA34*, *HHF2*, *CIN8* and *EGD1* genes were generated by PCR amplification of the *KanMX4* G418-resistance module. The resulting PCR fragments were transformed into GY59 strain already bearing pS-*ADE1* plasmid which is identical to wild-type 2 µ plasmid except for the *ADE1* insertion and therefore served as an indicator of plasmid loss in yeast strain by accumulation of red pigment. Disruption of these genes caused no significant effect on the 2 µ plasmid stability which was indicated by the formation of white colonies. pAFS60 carrying *STB* sequence was localised in mutant cells by fusing green fluorescent protein (GFP) to a DNA binding protein (lac repressor) on pAFS135 and incorporating binding sites (lac operator) for the GFP-lac repressor fusion protein on the pAFS60. In the presence of 2 µ plasmid (pS-*ADE1*), artificial plasmid (pAFS60) carrying the *STB* sequence can be stably maintained utilising Rep1 and Rep2 in *trans* at high copy number, although with less stability and at lower copy number than 2 µ plasmid. In conclusion, *HOS1*, *RME1*, *HHT2*, *RPA34*, *HHF2*, *CIN8* and *EGD1* genes are not involved the 2 µ plasmid stability.

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ABSTRAK

Plasmid 2 µ Saccharomyces cerevisiae adalah elemen DNA bukan kromosom berbentuk bulat yang stabil, wujud dalam bilangan yang banyak, dan tidak mempunyai kepentingan kepada hos. Selain daripada protein Rep1 dan Rep2 serta DNA STB yang terlibat dalam sistem kestabilan plasmid, protein yang dikodkan oleh kromosom yang juga terlibat dalam segregasi kromosom juga didapati mempunyai kesan yang sama ke atas segregasi plasmid. Oleh itu, tujuh gen yang sebelum ini pernah dilaporkan terlibat dalam kestabilan kromosom telah dikaji melalui penyingkiran gen. Fenotip yang terhasil daripada penyingkiran gen boleh memberi maklumat berguna mengenai fungsi sesuatu gen. Fragmen pemusnah untuk gen HOS1, RME1, HHT2, RPA34, HHF2, CIN8 dan EGD1 dihasilkan melalui amplifikasi modul KanMX4-G418 dengan menggunakan PCR. Fragmen PCR yang terhasil, digunakan untuk transformasi ke dalam strain yis GY59 yang membawa plasmid pS-ADE1, iaitu plasmid yang serupa dengan plasmid 2 µ yang asli, hanya berbeza dari segi penambahan ADE1, yang bertindak sebagai petunjuk kepada kehilangan plasmid daripada sel yis melalui penghasilan pigmen merah. Penyingkiran gen-gen ini tidak memberi kesan kepada kestabilan plasmid 2 µ, dibuktikan dengan pembentukan koloni putih. pAF60 yang membawa jujukan STB telah di kesan di dalam sel-sel mutan dengan menggabungkan green fluorescent protein (GFP) kepada protein pengikat DNA (lac repressor) pada pAFS135 dan memasukkan tapak pengikatan (lac operator) untuk protein gabungan GFP-lac repressor pada pAFS60. Dengan adanya plasmid 2 µ (pS-ADE1), plasmid buatan (pAFS60),yang membawa jujukan STB boleh dikekalkan secara stabil dengan menggunakan protein Rep1 and Rep2 di-trans dalam bilangan yang banyak, walaupun sedikit kurang stabil dan pada bilangan yang kurang daripada plasmid asli 2 µ. Kesimpulannya, gen HOS1, RME1, HHT2, RPA34, HHF2, CIN8 and EGD1 tidak terlibat dalam kestabilan plasmid 2 µ.

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