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SACCHAROMYCES CEREVISIAE. 2 µm PLASMID

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Abstract

A plasmid is an independent, circular, self-replicating DNA molecule that carries only a few genes, and exists as extrachromosomal genomes that can be inserted into a bacterial chromosome where they become a permanent part of the bacterial genome. This property gives you the ability to introduce genes into a given organism by using bacteria to amplify the hybrid genes that are created in vitro. A variety of Saccharomyces strains were examined for the presence of 2µDNA and, if present, for the pattern of fragments produced by its digestion with site-specific (restriction) endonucleases. Two strains were found that did not contain detectable levels of 2µDNA, and 2 strains contained 2µDNA molecules having only one EcoRI restriction endonuclease recognition site rather than the usual two.

Key words: Plasmid; EcoRI; Selfish DNA; Saccharomyces.

INTRODUCTION

The 2µm circle plasmid of Saccharomyces spp. was the first identified fungal cognate of bacterial plasmids. It is an extrachromosomal DNA species that has evolved molecular mechanisms to ensure its long term autonomous survival.

The yeast *Saccharomyces cerevisiae* contains approximately 100 copies of a 2µm circular DNA molecule. Guerineau, Grandchamp and Slonimski (1976) and Livingston and Klein (1977) have shown that 2µDNA contains an inverted repeated sequence comprising 20% of the total molecular lenght. These properties of 2µm DNA are interesting because of recent studies which show that inverted repetitions which form the boundaries of drug resistance genes on bacterial plasmids are important to the process of translocating the resistance genes from plasmid DNA to other cellular or phage DNA. The 2µm circle, a relatively small circular plasmid present in most common strains of *Saccharomyces cerevisiae*, has optimized a partitioning system and an amplification system that allow it to be propagated stably in a cell population at a copy number of approximately 60 to 100 per cell. The presence of 2µm plasmids in yeast species widely divergent from Saccharomyces spp. implies that their adaptive strategies for molecular parasitsm have been extremely successful over evolutionary time. The designation of the yeast 2 µm circle as a "selfish" DNA molecule has been confirmed by demonstrating that the plasmid is lost with exponential kinetics from haploid yeast populations grown in continuous culture. It has

been showen that plasmid-free yeast cells have a growth rate advantage of some 1.5%-3% over their plasmid-containing counterparts. This finding makes the ubiquity of this selfish DNA in yeast strains puzzling. Two other factors probably account for its survival. First, the rate of plasmid loss was reduced by allowing haploid populations to enter stationary phase periodically. Second, it was not possible to isolate a plasmid-free segregant from a diploid yeast strain. Competition experiments demonstrated that stability in a diploid is conferred at the level of segregation and that plasmid-free diploid cells are at a selective advantage compared with their plasmid-containing counterparts. Yeast cells in nature are usually homothallic and must frequently pass through both diploid and stationary phases. The 2 μm plasmid appears to have evolved a survival strategy which exploits these two features of its host's life cycle. The principal element in the strategy of 2 μm circle for persistence as a parasitic DNA species is its ability to raise its copy number. This propperty initially constituted a biological paradox. The 2μm circle is subject to strict cell cycle control of its replication. Replication of the plasmid proceeds bidirectionally from a single origin of replication. Essentially every 2 μm circle molecule (at least 95% of them) in a cell population acts as a replication template in every generation, and each undergoes one and only one round of replication. On the other hand, the 2 μm circle can be demonstrated to increase its copy number in several different situations. This requires that the plasmid be able to duplicate itself at a rate greater than once every cell cycle. How the plasmid can increase its copy number without abrogating stringent cell cycle control of replication posed an intriguing dilemma.

MATERIALS AND METHODS

Plasmids used in genetic engineering are called vectors. They are used to transfer genes from one organism to another and typically contain a genetic marker conferring a phenotype that can be selected for or against. Most also contain a polylinker or multiple cloning site, which is a short region containing several commonly used restriction sites allowing the easy insertion of DNA fragments at this location.

Plasmids are often used to purify a specific sequence, since they can easily be purified away from the rest of the genome. For their use as vectors, and for molecular cloning, plasmids often need to be isolated. There are several methods to isolate plasmid DNA from bacteria, the archetypes of which are the miniprep and the maxiprep. The former can be used to quickly find out whether the plasmid is correct in any of several bacterial clones. The yield is a small amount of impure plasmid DNA, which is sufficient for

analysis by restriction digest and for some cloning techniques. In the latter, much larger volumes of bacterial suspension are grown from which a maxi-prep can be performed. Essentially this is a scaled-up miniprep followed by additional purification. This results in relatively large amounts (several micrograms) of very pure plasmid DNA.

One common feature of all plasmids is a specific sequence of nucleotides termed an origin of replication (ori). This sequence, together with other regulatory sequences, is referred to as a replicon. The replicon allows a plasmid to replicate within a host cell independently of the host cell's own replication cycle. If the plasmid makes many copies of itself per cell, it is termed a "relaxed" plasmid. If it maintains itself in fewer numbers within the cell it is termed a "stringent" plasmid. Two different plasmids can coexist in the same cell only if they share the same replication elements. If they do not, they will be unable to be propagated stably in the same cell line, and are termed incompatible.

In nature, plasmid inheritance can occur through a variety of mechanisms. During conjugation between two bacterial strains, plasmids can be transferred along with the bacterial DNA, and this activity is controlled by a set of transfer (*tra*) genes that are located on the plasmid and not on the bacterial chromosome. The proteins produced by these transfer genes bind to the DNA at the ori site to form a DNA-protein complex known as a relaxosome. This complex makes a nick, or break, in one of the two strands of the double-stranded plasmid DNA molecule. The place where this break occurs is called the "nic" site, and the nicked DNA is said to be "relaxed" because the DNA unwinds as a result of the nick in one of the strands. The single-stranded DNA that is generated by the nick is thought to be unwound and transferred through the pilus, or mating bridge, that connects the two bacteria entering the recipient bacteria. The other strand is left in the donor bacteria. It acts as a template for the synthesis of a new complementary DNA strand forming a double-stranded plasmid DNA molecule.

Some nonconjugative plasmids can also be transferred into bacteria by means of a process called mobilization, as long as they carry the necessary (*mob*) genes. Others are taken up by bacterial cells during the process known as transformation. Finally, plasmids that exist in a host cell that undergoes fission (cell division) are simply divided between the resultant two daughter cells.

RESULTS AND DISCUSSION

One way of grouping plasmids is by their ability to transfer to other bacteria. *Conjugative* plasmids contain so-called *tra-genes*, which perform the complex process of conjugation, the sexual transfer of plasmids to another bacterium. *Non-conjugative* plasmids are incapable of initiating conjugation, hence they can only be transferred with the assistance of conjugative plasmids, by 'accident'. An intermediate class of plasmids are *mobilizable*, and carry only a subset of the genes required for transfer. They can 'parasitise' a conjugative plasmid, transferring at high frequency only in its presence. Plasmids are now being used to manipulate DNA and may possibly be a tool for curing many diseases. It is possible for plasmids of different types to coexist in a single cell. Seven different plasmids have been found in E. coli. But *related* plasmids are often incompatible, in the sense that only one of them survives in the cell line, due to the regulation of vital plasmid functions. Therefore, plasmids can be assigned into *compatibility groups*. Another way to classify plasmids is by function. There are five main classes:

- *Fertility*-F-plasmids, which contain tra-genes. They are capable of conjugation.
- *Resistance-(R)plasmids*, which contain genes that can build a resistance against antibiotics or poisons. Historically known as R-factors, before the nature of plasmids was understood.
- *Col-plasmids*, which contain genes that *code for* (determine the production of) bacteriocins, proteins that can kill other bacteria.
- *Degradative plasmids*, which enable the digestion of unusual substances, e.g., toluene or salicylic acid.
- *Virulence plasmids*, which turn the bacterium into a pathogen.

Plasmids can belong to more than one of these functional groups. Plasmids that exist only as one or a few copies in each bacterium are, upon cell division, in danger of being lost in one of the segregating bacteria. Such single-copy plasmids have systems which attempt to actively distribute a copy to both daughter cells.

Some plasmids include an *addiction system* or "post segregational killing system (PSK)", such as the hok/sok (host killing/suppressor of killing) system of plasmid R1 in Escherichia coli. They produce both a long-lived poison and a short-lived antidote. Daughter cells that retain a copy of the plasmid survive, while a daughter cell that fails to inherit the plasmid dies or suffers a reduced growth-rate because of the lingering poison from the parent cell.

Although plasmid DNA vectors have been extensively applied in biotechnology, there is still a lack of standard plasmid vector classification.

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