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Yeast Genetics in Analysis of the Organization of Eucaryotic Genomes

Genetika kvasca u analizi organizacije eukariotskih genoma

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Summary

Further progress in genetic manipulation of eucaryotes will depend largely on our understanding of the structure, organization and dynamics of eucaryotic genomes. Although the size of its genome is only about 0.5 % of the size of the human genome, the yeast ***Saccharomyces cerevisiae*** *has a remarkable place in these studies. It is not only an important model organism but is also used as a tool in the analysis of different complex genomes, including human. Genomic projects performed with yeast, like the whole genome sequencing, are also an important example for the organization of similar investigations in other organisms.*

Sažetak

Budući napredak na području genetičke manipulacije eukariota velikim će dijelom ovisiti o poznavanju strukture, organizacije i dinamike eukriotskih genoma. Iako je veličina genoma kvasca ***Saccharomyces cerevisiae*** *otprilike samo 0,5 % veličine humanog genoma, kvasac ima značajno mjesto u takvim istraživanjima. Osim što je važan modelni organizam, primjenjuje se i pri analizi drugih složenih genoma, uključujući i ljudski genom. Sekvencioniranje cjelokupnog genoma, kao i druga istraživanja provedena na razini genoma kvasca, dobar su primjer za organizaciju sličnih istraživanja u drugim organizmima.*

Introduction

The first transformation of yeast cells with exogenous DNA was reported in 1978 *(1,2)* and it marks the beginning of the molecular genetics of eucaryotes. After this initial success, the application of recombinant DNA (rDNA) techniques in order to manipulate eucaryotic genes was found to be much more complicated compared to bacterial genes. This is not surprising, considering the increased DNA content and complexity of eucaryotic genomes. For example, in sharp contrast with transformation of yeast cells, transformation of other eucaryotes is *a priori* mediated by illegitimate integration of foreign DNA *(3)*. This makes the gene replacement a difficult task, and the expression of integrated DNA highly dependent on transcriptional activity of the particular locus where integration occurred. Other problems may also arise from illegitimate integration of foreign DNA. Thus, it was observed that the genome containing several copies of a gene in ectopic (non-allelic) positions may pass through a sequence of events leading to the transcriptional silencing of the gene *(4)*. Such Repeat Induced Gene Silencing (RIGS) is of great biotechnological importance and indicates that the successful manipulation of eucaryotic genes should consider the genome as a whole and not only as an ensemble of coding and noncoding DNA. However, in spite of such problems, molecular genetics of eucaryotic cells is constantly gaining its place in modern biotechnology. This can be illustrated by the analysis of the topics presented at the VIth European Congress of Biotechnology held in Firence in 1993 *(5)*. Scientific presentations were organized in 93 sections dealing with specific areas of interest for biotechnology, out of which more than 2/3 at least partially relied on the use of rDNA techniques. For the first time, eukaryotic cells were slightly more represented in these studies than prokaryotic cells *(5)*.

One may predict that the genetic manipulations of eucaryotic cells will mark the evolution of different areas of biotechnology in near future and that such development will depend largely on our understanding of complex genetic interactions at the level of the genome. Following examples illustrate that the studies performed in the yeast *Saccharomyces cerevisiae*, in spite of its relatively simple structure and reduced genomic size, represent continuous contribution to our understanding of the organization of eucaryotic genome.

Chromosome structure and YACs

Yeast nuclear genetic material contains about 1.4×10^7 base pairs (bp) and is divided in 16 chromosomes bearing structural elements characteristic for an eucaryote: origins of replication (ARS), centromeres (CEN) and linear chromosomal ends – telomeres *(6)*. Whole chromosomes can be separated by gel electrophoresis and mapped by appropriate restriction enzymes *(7,8)*. Actually, the methods for electrophoretic separation of large DNA molecules were developed first with yeast protoplasts *(7)* and yeast chromosomes are routinely used as a size standard for electrophoretic analysis of complex genomes. Structural elements of yeast chromosomes have been isolated and are extensively studied giving valuable information about different aspects of chromosomal metabolism like, for example, telomere replication *(9)*. At the same time, by integrating these different chromosomal structural elements into a single plasmid molecule, a new type of cloning vector was constructed, called »Yeast Artificial Chromosome or YAC« *(10)*. The difference between YACs and other cloning vectors is that YACs may accommodate unusually large inserts ranging up to 2×10^6 bp while the size of inserts found in other vectors is limited to about 4.5×10^4 bp. Moreover, the mitotic and meiotic stability of the construct is increased with increased size of the insert. Therefore, YACs have become a valuable tool for the analysis of complex genomes. One of the most spectacular projects in todays genetics, physical mapping of the whole human genome, relies on cloning of the human DNA into YAC vectors *(11,12)*. Each clone gives a characteristic pattern on the Southern blot after hybridization with LINE (L1) probe *(12)*. The data are further automatically processed in order to arrange the clones containing overlapping sequences in linear »contigs«. This project is still in progress and is expected to significantly accelerate human genetic research.

Chromosomes in meiosis and recombination between repeated sequences

Cells that enter meiosis have both homologous chromosomes replicated such that each chromtide is present in four copies. A complex sequence of events ensures that haploid gametes formed at the end of meiosis contain the full set of chromosomes. The critical step, segregation of homologous chromosomes, is thought to be preceded and orchestrated by the formation of synaptonemal complex and subsequent recombination *(13)*. How are the homologous sequences recognized and the homologous chromosomes paired? In recent studies with yeast cells synchronized for meiotic division this process has been dissected in separate steps and analyzed by biochemical, cytological and genetic methods *(14-18)*. Special emphasis was put on the role of homologous recombination in chromosome segregation. It was found that the recombination is actually initiated before the formation of synapses and is not sufficient to assure proper segregation *(17,18)*. Experiments with YACs bearing inserts from different origins suggest that only those recombinational intermediates that are formed within specific chromosomal context help to orientate chromosomes for proper segregation *(17)*. An alternative, recombination-independent way of chromosome segregation was also described in yeast *(19,20)*.

These results raise the question of the actual mechanistic role of homologous recombination in meiosis *(21,22)*. One possibility is that the enzymatic machinery needed for genetic recombination is involved in two distinct, temporally separated steps of chromosomal metabolism during the first prophase. During leptotene, early prerecombinogenic structures like double-stranded breaks or single-stranded tails, may perform the genome-wide search for homologous sequences. Stable synaptonemal complex is formed only in the presence of extended homology, in which case recombination may proceed to form crossovers needed for chromosome segregation. Genes introduced in the yeast genome in ectopic positions recombine during meiosis as efficiently as allelic homologues; moreover, recombinational intermediate is frequently resolved as crossover, giving rise to reciprocal recombinations *(23)*. The same was observed for mitotic recombination, indicating that the genome may become significantly destabilized by the presence of dispersed homologous sequences. Naturally occurring repeated sequences are stable only if they are under the specific control for recombination, like yeast rRNA genes, or if they have accumulated enough mutations to escape homologous recombination. This applies only for the sequences longer than 250 bps; shorter homologous fragments do not recombine efficiently in either allelic or ectopic position *(24,25)*. These observations are very helpful for our understanding of the organization of more complex eucaryotic genomes, containing lot of noncoding DNA within introns or as repetitive DNA *(26)*.

Sequencing of the yeast genome

Although the laboratory methods for deducing the primary structure of DNA molecules have been available for more than 15 years, the sequencing of the whole human genome (3×10^9 bp) still represents the most challenging project in modern biology. Some of conceptual and organizational controversies that accompany this enterprise from its beginning are successfully resolved in another genome sequencing program, that of the yeast *Saccharomyces cerevisiae*. This project, supported mainly by the European Biotechnology Program, started a few years ago and the whole sequence will be known by the end of 1996 *(27)*. Today, about one half of the sequence is already read including the complete sequences of the chromosomes II and XI *(28,29)*. These data represent very valuable source of information about eucaryotic genes, gene products and chromosomes. For chromosome III, 171 probable gene products were designated, 61 % of them having significant sequence similarities in the current databases. As many as 54 % have already known functions or are related to functionally characterized proteins, allowing partial prediction of protein function *(30)*. For some other genes, the possible function of the gene product can be deduced from the phenotype of the null mutant created by gene disruption. Moreover, these data make possible the analysis of the structure and organization of eucaryotic chromosome on a new level. For example, for both chromosomes analyzed so far it was found

that they contain (G + C)-rich peaks regularly distributed along two chromosomal arms *(29,31)*. Surprisingly, the third base of codons in open reading frames gives the major contribution to this distribution. The actual role of this high-ordered sequence organization along the chromosome is not known, but different predictions could be tested by introducing modified chromosomes back into the yeast cell.

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