MICROBIAL GENETICS

Yeast Genetics

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CONTENTS

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Yeast as a model eukaryote, Yeast cell cycle, Mitotic and meiotic divisions, Mating type, Mating type switching, Cell cycle control, Random spore analysis, Tetrad analysis, Extra chromosomal inheritance, Yeast genome.

Yeast as an organism of choice

Yeasts belong to the larger category of fungi, which are spore bearing microorganisms with absorptive nutrition, no chlorophyll and mode of reproduction as sexual as well asexual. These are facultative anaerobes and can obtain energy by fermentation (in the absence of oxygen) as well as through oxidative metabolism. Such a biochemical capacity leads to ethanol production by yeasts which is commercially a successful process. The ability to grow in the absence of oxygen also allows for isolation of mutants that are impaired in mitochondrial functions and hence unable to assimilate non-fermentable carbon sources such as glycerol, lactic acid etc. In fact, a large amount of information on the respiratory enzymes was gathered by working on such 'petite' mutants.

Yeasts are eukaryotic and hence more complex than bacteria. The DNA is organized into 16 chromosomes (haploid number). The ease with which mutants can be isolated, characterized and mapped makes it an ideal system to carry out eukaryotic genetics. The pace with which the work can be carried out is as rapid as with bacterial systems. Some of other properties that make it useful are its single cell nature, non-pathogenicity, ease of cultivation, rapid growth (the doubling time on glucose is 90 min and about 3.5-4 h on a non-fermentable carbon source), application of replica plating methods, mutant isolation and a well-defined haploid and a diploid life cycle. Various stable biochemical mutants can be isolated, many of them for essential functions. Baker's yeast cells are also available commercially and provide a cheap source of the strain.

As described in the later sections, the life cycle of yeast is perfect for carrying out classical genetic studies. It exists in a stable haploid as well as a diploid state. The mutants can be conveniently isolated and expressed in the haploid state and complementation tests carried out in diploids to study the segregation of genes. Since the products of mitotic division are held together in an 4- spored ascus, the analysis of their phenotype gives a useful tool for following segregation of markers.

Two significant findings have led to revolutionary advances in application of modern molecular biological techniques to yeast. These were the 'discovery' of the 2μm plasmid, the origin of replication of which was used for construction of numerous stable plasmid vectors. The other was the development of a transformation system in *Saccharomyces cerevisiae*. During early years, a large number of structural genes were identified from plasmid libraries by complementation analysis. Many of the plasmids (Integrative YIp series) could integrate into yeast chromosome by homologous recombination. External DNA containing partially homologous sequences can therefore be directed to specific locations in the yeast genome. Coupled with the high levels of gene conversions noticed in yeast, these have led to direct replacement of engineered sequences into their normal chromosomal locations. In fact, a library of mutants for each of the annotated genes was constructed at Stanford University. These single gene knock-out libraries are available through commercial sources for use by the scientists world over.

Very recently, transformations with single synthetic oligonucleotides have been reported in yeasts allowing manipulations of genes encoding proteins. This technique has been exploited for studying gene regulation, structure- function relations, chromosome structure etc. A number of

mammalian proteins are also being expressed in yeast to study their functions. With the availability of the genome sequence of *S. cerevisiae,* a new way of doing science in this yeast has emerged.

Yeast cell

The structure of a typical yeast cell is shown in Fig. 1. It is characterized by the presence of cell wall to which the cell membrane is attached strongly. These are unicellular and most of these are classified under Ascomycetes. The cells are usually spherical, oval, cylindrical, and cell division occurs generally by budding. Some yeasts such as *Schizosaccharomyces pombe* divide by fission and thus are called as fission yeasts. The size of a typical cell varies from 10µm 20µm which is much larger than the bacterial cell and therefore yeasts can be easily distinguished from bacteria microscopically. The presence of internal structure such as nucleus also is a distinguishing feature. It has all the characteristic organelles of a typical eucaryote, namely nucleus, golgi bodies, lysosomes, mitochondria etc. Many yeasts exhibit sexual reproduction through mating between opposite mating type cells. The resulting zygote undergoes meiosis. The details are provided in sections below. Many yeasts are dimorphic and can exist in both filamentous as well as spherical shapes. Such changes are dependent on temperature, CO_2 concentration, and nutritional status of the yeast. Certain characteristics are exhibited only by the filamentous form. The filamentous form of many yeasts such as *Candida albicans* is pathogenic.

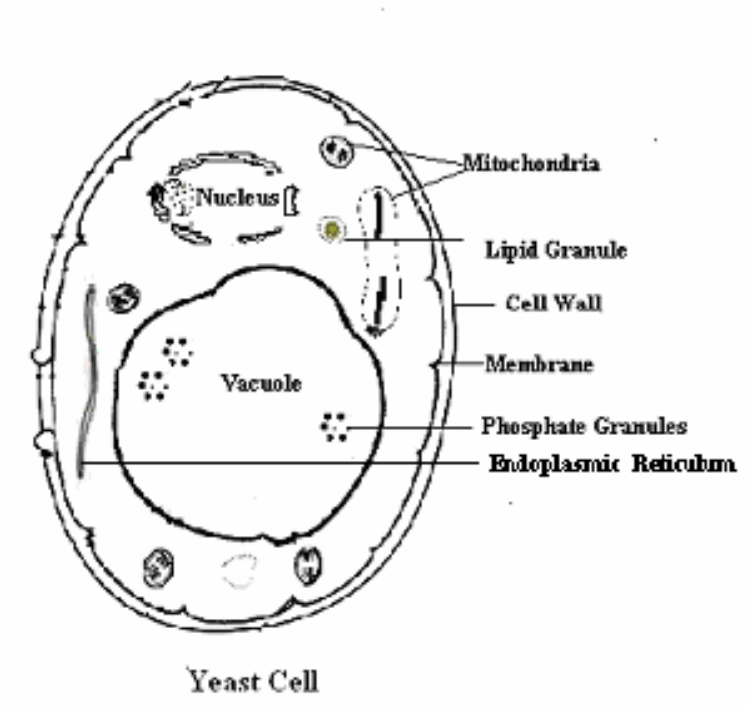


Fig. 1: Structure of a typical yeast cell showing various organelles

Life cycle of yeast

The cell cycle of a typical yeast *S. cerevisiae* is shown in Fig. 2. The entire genetic material of *S. cerevisiae* is organized in the form of distinct chromosomes. The yeast cells can exist as stable haploids (ploidy: n, one set of chromosomes) or diploids (2n, two sets of chromosomes). In *S. cerevisiae*, the haploid set of chromosomes is 16 in number (32 in case of diploid set). The DNA in these chromosomes varies in size from 200 to 2,200 kbp. The total sequence is of 12,052 kbp in which about 6000 open-reading frames (of over 100 amino acids) have been identified. About 5800 of these were correctly predicted to correspond to protein coding genes, and thus could be assigned a function. The yeast genome is considered to be tightly packed with 72% representing genes, a fact reminiscent of prokaryotic genome organization. On the other hand, in contrast to higher eukaryotes wherein a large amount of repetitive DNA, for which no specific functions has been assigned as yet, are found. The average size of the genes is 1.45 kbp or about 480 codons within a range of 40 to 4910 codons. Yeast genes are intron-free and only about 3.8 % contain introns. About 30 % of the genes have been characterized experimentally (Sherman, F., Introduction to Yeast Genetics….., available on-line) and of the remaining 70 % about one-half either contain a motif of a characterized class or correspond to genes coding for proteins that are structurally related to characterized gene products from yeast or from other organisms.

Two sets of markers or genes are present in the diploid cell, one on each of the homologous chromosomes. The term *allele* refers to the different versions of the same gene present on homologous chromosomes. If the allele on one of the two chromosomes produces a defective gene function, its function can be supplemented by the normal copy, hence the effect may be masked. In this case, we call the mutant copy as *recessive*. Else, if it does show its effect we call it *dominant*.

Being able to exist both as haploid and diploid forms, the life cycle of yeast can be described following mitotic and meiotic divisions. In the mitotic division, chromosomes undergo doubling and the products are distributed uniformly to the resulting daughter cells. This state can continue indefinitely, as shown in the Fig. 2 (bold face). On the other hand, a diploid cell (arising out of a fusion of *a* and α mating type) can be induced to undergo meiosis to produce haploid ascospores/progeny (represented as --- in Fig. 2). Meiosis involves two divisions. During the first division, the two sets of homologous chromosomes are segregated to two daughter cells and the chromosome number is halved. During this process, genetic exchange also occurs due to crossing over events. The second meiotic division is actually mitotic in nature and no change or recombination event occurs here. Consequently, there are four products of the meiotic division. In yeasts, the four cells of meiotic division are held together in a sac like structure, called the ascus. The name Ascomycete is derived from these features. These cells are called as ascospores and are useful in carrying out genetic analysis. For many microorganisms, including yeasts, the gametes (or the ascospores) are haploid, look identical and in spite of having two different mating types ($2a$, 2α), these cannot be distinguished physically. The cells of two different mating types can come together to form diploids (Fig. 2).

If the sets of alleles carried on a pair of homologous chromosomes are identical in the diploid, the cell is said to be *homozygous* for these markers (or genes). The resulting haploids due to meiosis of such cells will also be identical for those genes. However, if the corresponding set of alleles differs in the two chromosomes, the diploid is *heterozygous*. The four haploid cells

resulting out of such diploids will not be identical for those set of alleles. In a heterozygous diploid, the trait that shows its phenotype is controlled by dominant allele and one which does not show up is the recessive allele.

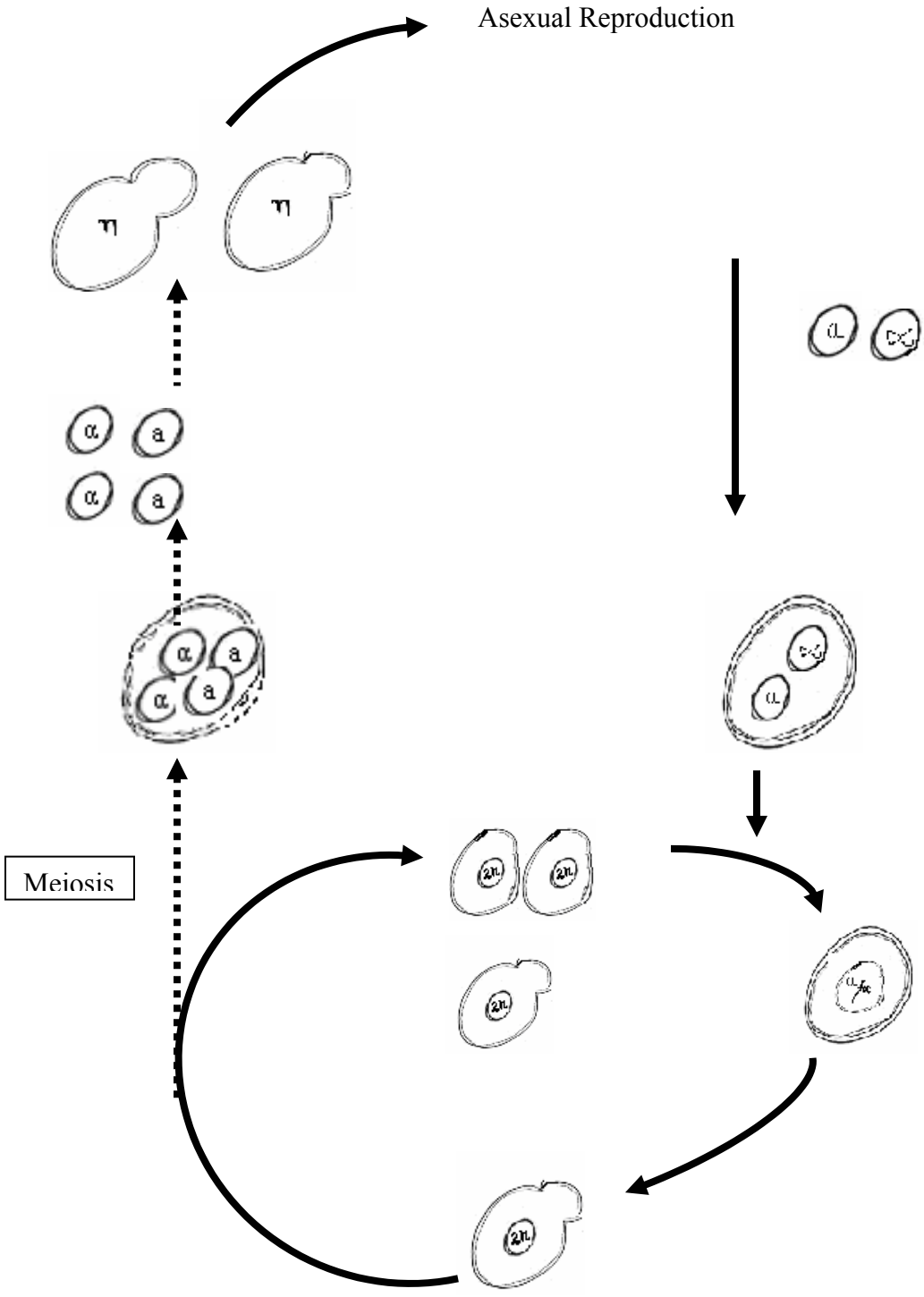


Fig. 2: Life cycle of the yeast *Saccharomyces cerevisiae*. Both haploid and the diploid states can be maintained stably

Cell cycle

Yeast has served as the most important model system to unravel the molecular events and their regulation as a cell prepares itself to undergo mitotic division. The control of mitosis or cell division is a reflection of 'Cell cycle events'. Every cell has a periodicity in which it has to undergo a precise series of molecular events such as replication, synthesis of proteins, followed by cell division and culminating in the appearance of two daughter cells, which are identical to the mother cell. The period between the two successive divisions earlier called as the interphase is now expanded into a cell cycle. Fig. 3 illustrates the different phases in the eukaryotic cell cycle that defined a phase of intense metabolic activities. At the outset of a division, is the G1 phase (also the first Gap). This is characterized by synthesis of RNAs and proteins but no DNA synthesis occurs. After G1, starts the S phase, characterized by DNA synthesis (i.e. chromosome duplication). This phase lasts until all the chromosomes have doubled, that is they consist of two chromatids each. The period from the end of the S phase till mitosis is the G2 phase (the second Gap). M phase is where actual mitotic division occurs resulting in two complements of the chromosomes. The synthetic activities come to a complete halt during the M phase. The whole process of mitosis occupies only a small part of the cell cycle and the lengths of S and G2 phases are also fairly constant among different cell types. Most variation is seen in the length of time spent in the G1 phase. Sometimes late in the G1 phase, cells are either committed to initiate DNA synthesis and complete the cycle or withdraw from the cycle to enter a resting phase called G0. Readers may remember that cancer cells avoid entering G0 or pass through it quickly so as to remain ever proliferative. In *S. cerevisiae*, the S phase is quickly followed by mitotic (M) phase, so the length of the G2 phase is short or absent. Also, since bud is formed, there is unequal cytoplasmic division. Some unusual features are exhibited by yeast during mitosis in that the nuclear membrane does not completely break down and thus segregation of chromosomes occurs within the nucleus. In rapidly growing yeast cells (say, on glucose) the whole cycle takes about 90 min.

Two commitment points are recognized in the cell cycle:

- Commitment to chromosome replication: this occurs in the G1 phase. If conditions are satisfactory for a passage through this phase, a cell enters into the S phase. In yeasts, this point is called as a START point.
- Commitment to mitotic division: this occurs at the end of the G2 phase. If a cell does not divide at this point, it remains in the condition of having two complements of the chromosomes.

In addition to these, a fine-tuning of the cell cycle also occurs through various checkpoints, which ensure that the next phase will occur only on satisfactory completion of the previous step. Many of these points oversee that the integrity of the chromosomes is maintained.

An insight into the molecular events occurring during the cell division was provided by isolation of mutants that are defective in the same. Since such mutants are not expected to survive under normal conditions, only conditional mutants such as those able to grow at permissive temperatures were isolated. Thus, at lower temperature, these cell division cycle (*cdc*) mutants would survive and were called as temperature sensitive mutants. Since these mutants affect the morphology of the cell, which can be visualized under a microscope, various mutations can also

be linked to distinct phenotypes. Due to the ability to carry out complementation analysis in diploids, mutants could be individually placed in different loci. About 800 genes are involved in the cell division process but not all are required for regulating the cell cycle. Many of these control ancillary steps that feed into the cell cycle.

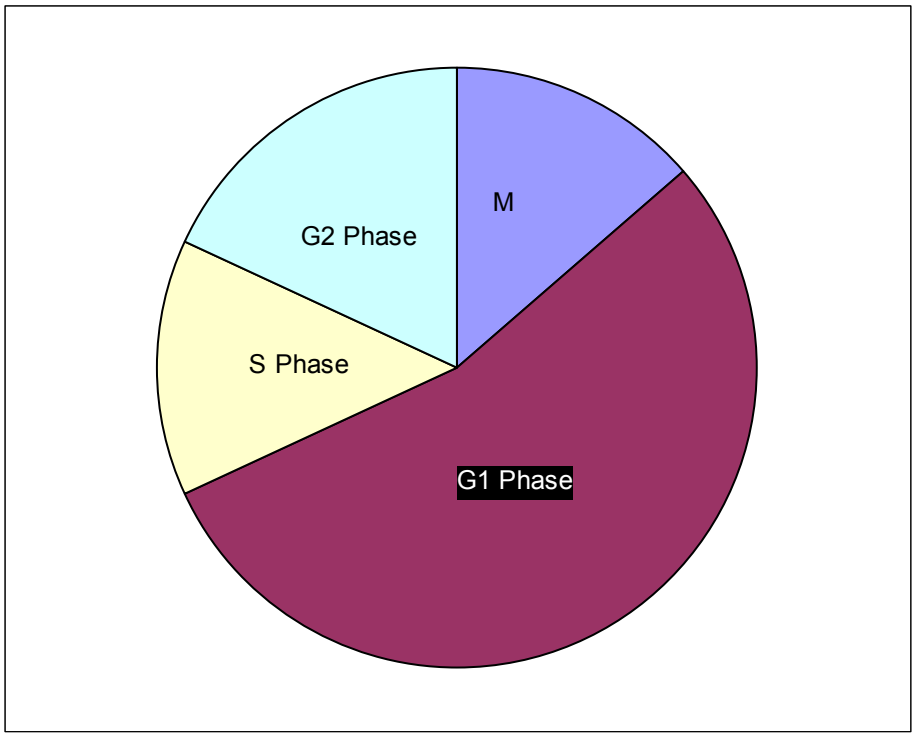


Fig. 3: The eucaryotic cell cycle. The consecutive cycles comprising of G1, S, and G2 phases are separated by the mitotic (M) phase. Cells may withdraw from the cycle due to mutations or may re-enter into it.

The two committed phases, described above are critically regulated by *cdc2* (in *S pombe*) or its equivalent *cdc28* in *S. cerevisiae*. The regulatory network of proteins, however, comprises a smaller sub-set of gene products. There are four classes of these proteins: Cyclins (Cln1,-2, and –3 and Clb1,-2,-5, and –6, which bind to kinase Cdc28); the inhibitors, degraders, and competitors of cyclin/Cdc 28 complexes (Sic1, Cdh1, Cdc20, Cdc14); transcription factors (SBF,MBF,Mcm1/SFF,Swi5); and, check points (the cell size, DNA replication and damage, spindle assembly). Cyclins are the key regulatory components of hetero-dimeric kinases, the levels of which increase or decrease as the cells progress through various stages of cell cycle. The catalytic subunits of these kinases, called the Cyclin-dependent kinases (CDKs), have no kinase activity unless associated with the Cyclins. The different cell cycle phases (G, S, M) produce their own Cyclins, which in association with CDKs control the passage of the cell through the cell cycle. If at the check-point preceding the S phase, all events are normal, G1 Cyclin- CDK complexes are expressed. These prepare the cell for DNA synthesis by *activating* transcription factors that promote (i) transcription of DNA replicating enzymes and (ii) the transcription S-phase Cyclins and CDKs.

The activity of the latter two proteins is kept under check initially by inhibitors. Late in G1, the G1-cyclin-CDK complexes also induce degradation of inhibitors by phosphorylating them and stimulating their polyubiquitination by multi-protein SCF ubiquitin ligase. The active S-Phase Cyclin-CDK complexes phosphorylate regulatory sites in the proteins that form DNA pre-replication complexes (which are assembled during G1), which allows DNA synthesis to proceed. This process occurs only once so that only one round of DNA synthesis can occur during the S-phase. During this phase, mitotic Cyclin-CDK complexes are also synthesized but kept under check by phosphorylation at inhibitory sites until DNA synthesis is completely over. Dephosphorylation at the inhibitory sites activates these proteins which phosphorylate multiple proteins that promote chromosome condensation, disaggregation of the nuclear envelope, laying of mitotic spindle etc. Hence, the events of mitosis ensue resulting in division of the chromosomes followed by their partitioning into the daughter cells. The events of mitosis also induce degradation of spindle proteins. This degradation of proteins is mediated by ubiquintinylation. Late in anaphase, the anaphase-promoting complex directs polyubiquitination of several proteins (including mitotic Cyclins) and their subsequent degradation. The decrease in mitotic CDK activity allows constitutively active protein phosphatases to remove the phosphates added to specific proteins by the mitotic Cyclin-CDK complex. As a result of this, reversal of steps of mitosis occur in which the nuclear envelope, Golgi apparatus are reassembled. The cytoplasm divides resulting in the formation of daughter cells.

Passage through various phases of the cell cycle is thus regulated by a number of proteins that control various transition points G1 $\rightarrow$ S phase $\rightarrow$ metaphase $\rightarrow$ anaphase$\rightarrow$ cytokinesis. The steps are irreversible as these are triggered by regulated degradation of proteins. Thus the process of cell division is unidirectional.

Cell division

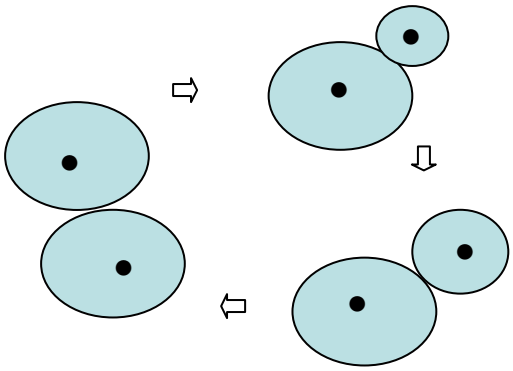
Mitosis finally culminates into cell division so that the two nuclei formed after division so that the two nuclei formed after division are distributed into two daughter cells. In yeast, cell division is also the basis of the asexual mode of reproduction. Such a division can occur through budding (budding yeast such as *S. cerevisiae*) or fission (fission yeast, *S. pombe*). In budding yeast (Fig. 4 A), a small offspring cell appears on one side of the mother cell which grows in size. Physical separation of the daughter cell may not be immediate and has to wait until the chromosomes have completely divided. One set of chromosomes (enclosed in the nucleus) moves to the daughter cell followed by physical separation. This leaves behind a 'bud scar' on the mother. Several of these can be seen on the mother cell. In the fission yeast, the cell grows longitudinally (Fig. 4 B) to a length of about 14-16 μm and then divides equally to generate the daughter cells (7-8 μm).

Cell ploidy

In the previous sections, the terms haploid, diploid have been defined. Tetraploids are yeasts that contain four sets of chromosomes. Many of the industrial strains used for brewery purpose are tetraploid in nature. Due to the presence of multiple copies of genes, involved in industrial processes such as alcohol, acid production, polyploids are very often preferred in the industry. In general, yeasts tolerate haploidy, diploidy, tetraploidy quite well and no biochemical disorders

are noticed as a consequence of multiplicity of ploidy. However, if a biochemical phenomenon needs to be studied, it is desirable to isolate the mutants for the same in haploids due to dominance/recessiveness of the gene in diploids.

A.



B

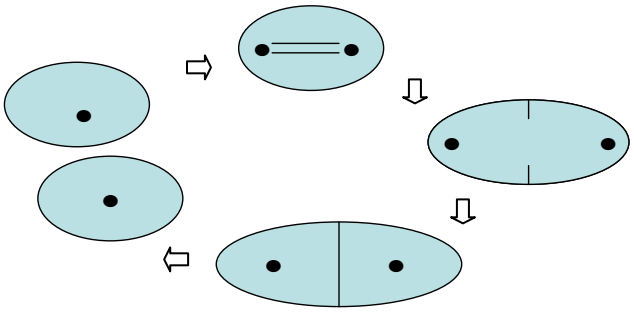


Fig. 4: Cell division of a budding yeast *Saccharomyces cerevisiae* (A) and a fission yeast *Schizosaccharomyces pombe* (B)

Infrequently, the cells may form that have more or fewer than the normal number of any chromosome. These are called as aneuoploids and form when a pair of chromosomes fails to separate during the process of meiosis. This phenomenon is called as chromosome non-

disjunction. It may also occur when chromosomes lag and move slowly than the others during anaphase and are excluded from the telophase nucleus and are lost.

Sexual reproduction

Yeast has a simple sexual reproduction pathway, wherein a diploid cell undergoes meiosis to generate haploid cells/ascospores. These haploid ascospores then develop into gametes necessary for sexual reproduction. For yeast to enter meiosis, three specific nutritional environment, such as lack of glucose, nitrogen source (or any other essential nutrient) and presence of a non fermentable carbon source, must exist. Moreover, yeast is heterothallic so that the two gametes must be different in their mating type status, as described below. Essentially, when all the conditions are fulfilled, the two gametes fuse to produce the zygote. And, the zygote can undergo meiosis to produce the ascospores as described above.

Mating type a and alpha

As discussed in the previous section, the two haploid gametes can undergo mating if they have two opposite mating types called as *a* and *α* type. The two forms are indistinguishable except for the mating behavior. The cells of *a* type mate with *α* type cell or vice-versa. This characteristic is determined genetically. While majority of the cells are stable in either *a* or *α* type, some strains are able to *switch* the mating type from *a* to *α* or vice-versa. This may result in diploid formation (a/*α*) from a pure *a* or α type of culture.

The switch in the mating type occurs due to a directed transposition event that occurs in the yeast strain carrying HO gene. The mating type behavior is controlled by the mating type (MAT) locus which can either be MAT*a* or MATα. Elsewhere on the yeast genome are additional copies of both the '*a*' and `*α'*, which are silent i.e. not expressed. These 'silent genes' serve as the source of the gene that can replace 'much like a cassette' the information at MAT locus. Thus, MAT*a* can be converted to MATα and vice-versa. Mating type switching allows the generation of cells with different mating type that can consequently mate resulting in the formation of ascospores (Fig. 5).

The determination of mating type is carried out by gene specific products. Both *a* and *α*, among other genes, regulate the production of the peptide hormones called *a* factor and *α* factor respectively. These peptides are excreted into the medium of yeast cells. The hormones bind to the surface of the opposite mating types and bring about morphological changes so that the cells can associate and fuse. It appears that *α* cells have receptors only for *a* factor and similarly *a* cells for *α* factor. The association of these two types of cells brings forth the events of cell fusion, or sexual reproduction.

Yeast genetics

A large body of information is available on the molecular genetics of the yeast *S. cerevisiae.* This is due to the ease with which it can be cultivated in laboratory and its accessibility to mutational studies. Some principles of yeast genetics are described below.

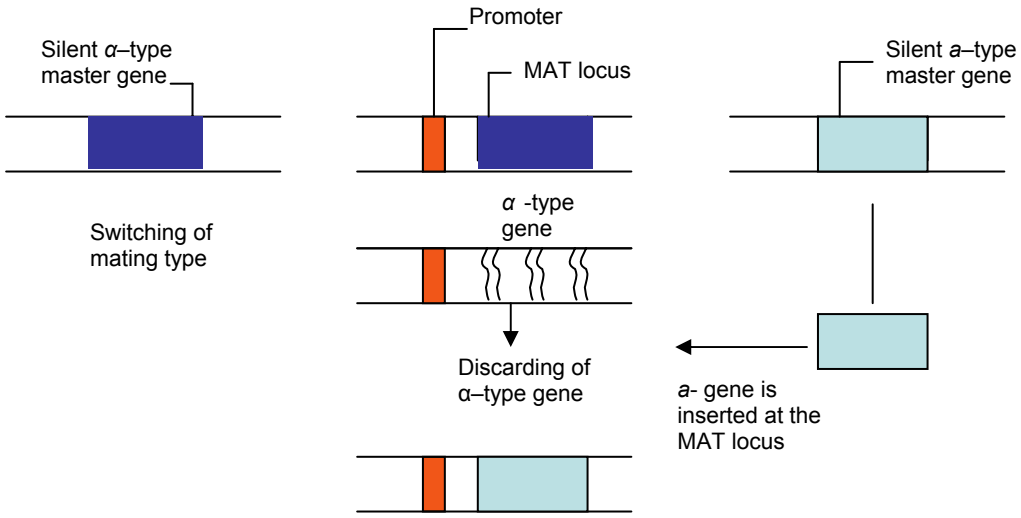


Fig. 5: Switching of the mating type in *S. cerevisiae*. The gene that is inserted at the MAT locus determines the active mating type. The mechanism is similar to insertion of a cassette. The old copy at the MAT locus is degraded

Genetic nomenclature

All genes are given three letter names with numerals following them, such as *CAN1*. Classically, these indicate the phenotype of the cell. Thus *CAN1* is for canavine resistance or *URA3* refers to a gene involved in uracil biosynthesis. Genes can also be named after the proteins or RNAs they encode, with these being shown in capital, for example, *CMDI* coding for calmodulin. Loss of function in the mutant is shown in italic font and in the example discussed above, mutations at different locations in the same genes are shown as *ura3-1* or *ura3-33*. Phenotypes are usually indicated as follows: STE^{+} and TS^{+} stand for not sterile and not temperature sensitive. On the other hand, ts^{-} and ste^{-} stand for temperature sensitive and sterile. The `+' sign means the normal (wild-type) and `-' stands for the loss of or alteration in that character.

Chromosomal Inheritance

The pattern of gene inheritance through chromosomal segregation can be followed very easily in *S. cerevisiae*. Since the four products of meiosis are held together in the ascus, these can be isolated and analyzed. This is called tetrad analysis. For the spores to be formed, it is necessary

that the cells of the opposite mating type come together to form a diploid, and the diploid zygote undergoes meiosis to produce ascospores. For this, the diploid *a/α* should be cultivated on sporulation medium (Table 1) which induces meiosis.

The number of genes responsible for the trait can be determined by carrying out analysis of the meiotic products. The asci containing the spores are digested with cell wall lysing enzymes and the spores are separated using a micromanipulator. These are placed on a complete medium where the spores will germinate producing vegetative strains of both *a* and *α* mating types. The phenotype of the spores can be determined by standard techniques like replicating plating. Thus, the segregation of genes (or appearance of a trait) in the progeny can then be followed.

Table 1: Composition of the sporulation medium

Sporulation medium	
Potassium acetate	10 g
Bacto-agar	20 g
Distilled water	1000 ml
This medium directly induces sporulation in auxotrophic diploids without allowing for division. It takes 18-24 h for the diploids to sporulate	

Random spore analysis and Tetrad analysis

In yeasts, there are two methods available to follow the pattern of linkage and inheritance. These are *random spore analysis* and *tetrad analysis*. In the first method, spores are randomly collected from the ascus upon digestion with cell wall lysing enzyme and allowed to germinate on the nutrient medium. The resulting culture is analyzed for phenotypic characteristics. The purpose of random spore analysis is to follow the map distance between various markers. For instance, crossing between two parents with three linked/unlinked genes can be set up to determine the segregation of the three genes. If these are linked, these will co-appear in the progeny at a frequency that reflects their distance (Also, note that here progeny, not gametes, are being produced). The haploid nature of the ascopores simplifies the task of following segregation. When the diploid undergoes meiosis, the two parental type phenotypes e.g., (+ + +, a b c) are produced if no crossover occurs during meiosis (Fig. 6). This is due to the fact that genes are either far apart on the chromosome or are on different chromosomes. For the genes located close by, two more classes, viz., single cross-over (SCO) and double cross-over (DCO) will appear. Thus, such a cross allows one to determine linkage relationships and construct linkage maps.

In *tetrad* analysis, since the four products of meiosis (the first meiotic and second mitotic division) are held together, analysis of these allows determining the linkage relationship between the genes. In a cross between a haploid with two mutations (a b) and the wild type (+ +) , three

types of asci can be obtained as shown below in Fig. 7. These are called as parental ditype (PD) since the spores have parental (a b, + +) phenotypic characteristics. The non-parental ditype (NPD) contain spores that do not resemble (a +, + b) the parental type and are recombinants. The tetratype (T) ascus has four types of spores: two resembling the parental phenotype (a b, + +) and two recombinants (a +, + b). The existence of the recombinant phenotypes indicates that a crossover event has occurred at the four strand stage of meiosis.

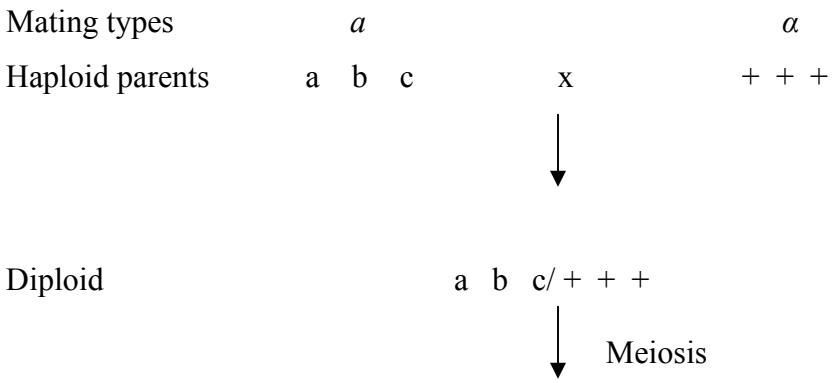
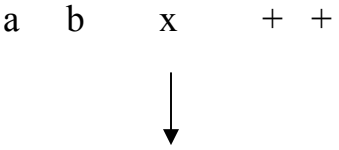


Fig. 6: A three gene cross being analyzed by random spore analysis



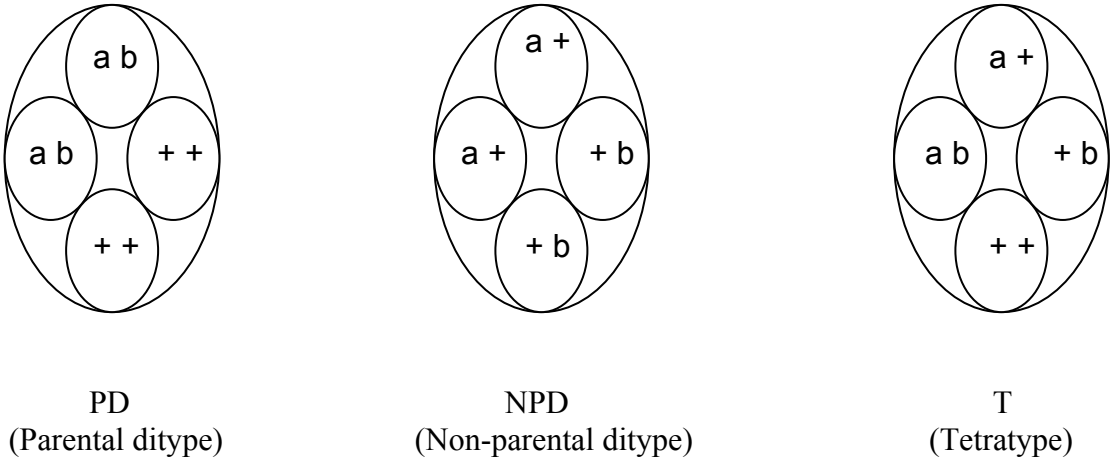


Fig.7: Different types of asci resulting from a cross between mutant (a b) and wild type (+ +) cell

In the event that the two genes are on separate chromosomes and at least one gene is not centromere-linked or if two genes are widely separated on the same chromosome, there is independent assortment and the ratio of PD:NPD:T is 1:1:4. If two genes are on different chromosomes and are linked to their centromeres, there is a reduction of the proportion of the T asci (PD : NPD : T = 1:1:<4). If two genes are linked, there will be greater proportion of PD to NPD asci. For such linked genes, the distance can be estimated by calculating the percentage recombination.

$$100 \times \frac{\frac{1}{2}(T) + NPD}{\text{Total tetrads}} = 100 \times \frac{\frac{1}{2}[T - 2NPD] + 4NPD}{\text{Total tetrads}} = 100 \times \frac{\frac{1}{2}T + 3\,NPD}{\text{Total tetrad}}$$

This calculation is based on the fact that T type of tetrad which represents single crossover (SCO) has only half the spores of recombinant type and is also generated by two events of double crossover (DCO of 3-strand double type). Also, the DCO class produces three types of patterns (PD, T and NPD) and its estimates can be derived from the relationship 4 × NPD. These double crossover events and their outcome are described below and explained by Fig 8.

Alternatively, better mapping functions can be derived if spores in these tetrads are taken into consideration as crossover products. Thus, 4NPD will have 4 crossover and T-2NPD will represent 2 crossover products. The relationship now will be:

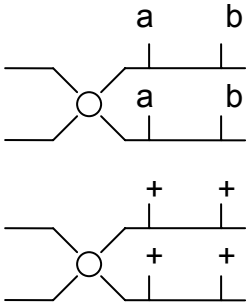
$$100 \times \frac{(T - 2\,[NPD]) + 4\,(NPD)}{\text{Total tetrads}} = 100 \times \frac{T + 6\,(NPD)}{2(PD+NPD+T)}$$

In the event of a double crossover, as described, four types of progeny will result (Fig 8) depending on if it is a 2- strand double crossover (Fig. 8C.1), 3-strand double crossover (Fig. 8C.2) or a 4-strand double crossover (Fig. 8C.3). Since the only unique class is the NPD, the frequency of all double crossovers, assuming no chromatid interference, can be inferred to be 4(NPD). In such a case, correction for a single crossover has to be made by subtracting the double crossovers that yield tetrads- hence T- 2 (NPD). In case segregation of two genes located far away on the chromosomes is being followed, a number of assumptions regarding centromere interference are to be made. Thus, the only accurate way of measuring long intervals is by the summation of shorter intervals. Also, if more than two markers are being followed, the data should be analyzed by considering only two genes at a time.

In fungi, the distance between a gene and its centromere can also be measured (provided known centromere-linked genes are present in the hybrid) by the frequency of second-division segregation (SDS) which is analogous to the frequency of T asci of two gene markers. A gene located close to the centromere, such that no crossing over is possible in this interval, will result into segregation of two alleles at the first meiotic division itself. However, if a gene is located at some distance from the centromere, a crossing over may occur and the two alleles will segregate at second meiotic division (Fig 9). Since crossing over is a measure of genetic distance, the distance in centiMorgan (cM) is equal to:

$$100 \times \frac{1}{2}\,\frac{\text{SDS tetrad}}{\text{Total tetrads}}$$

A. No cross over

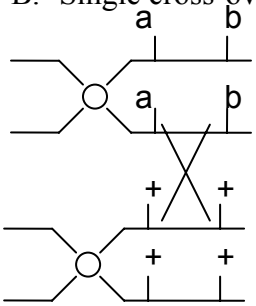


Asci type

a b
a b
+ +
+ +

PD type

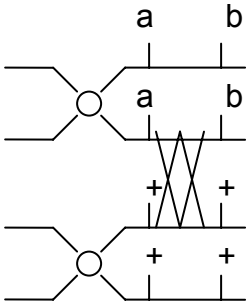
B. Single cross-over event



a b
a +
+ b
+ +

T type

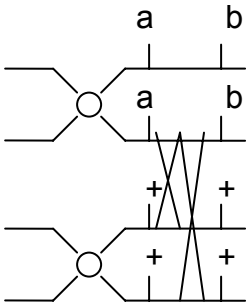
C. Double cross-over event



a b
a b
+ +
+ +

PD type

(1. Two-strand double cross over)

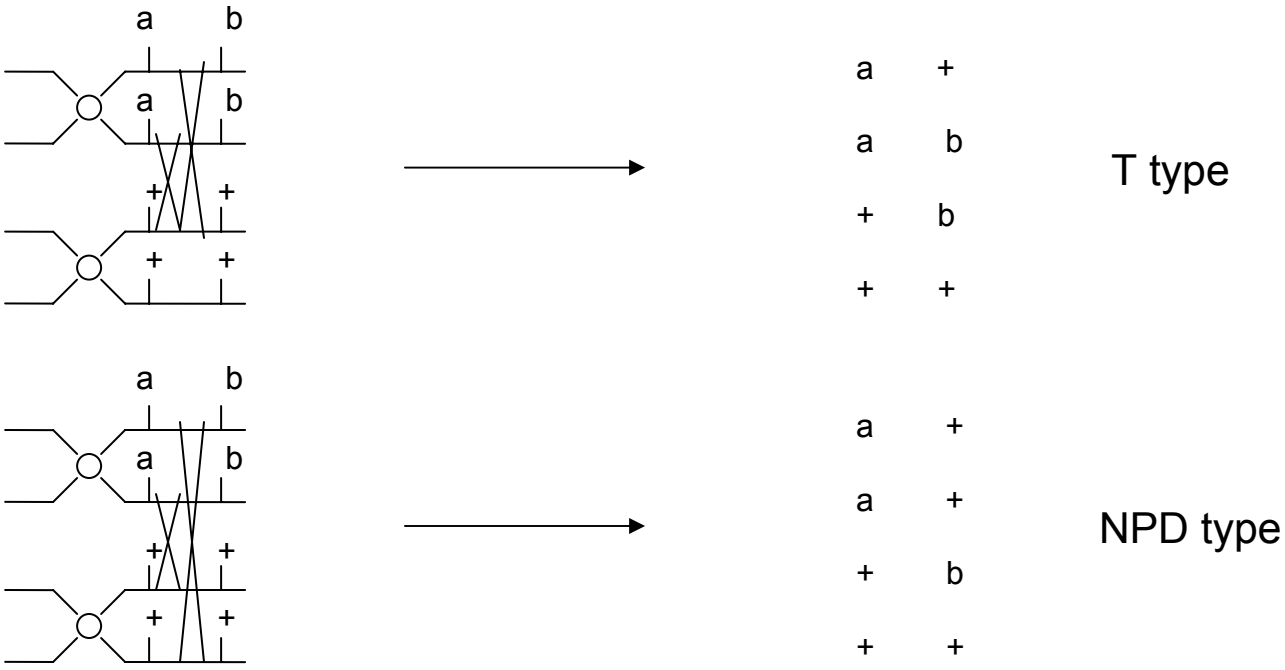


a b
a +
+ b
+ +

T type

(2. Three-strand double cross over)

or



(Four strand double crossover)

Fig. 8: Analysis of tetrads for a cross between the parents a b x + + when both the genes are located on the same chromosome. If there is no cross over (A), PD asci will result; in the event of a single crossover (B), T type asci will result; in the event of a double crossover event (C), PD, T or NPD asci result depending upon the chromatids involved in the exchange event.

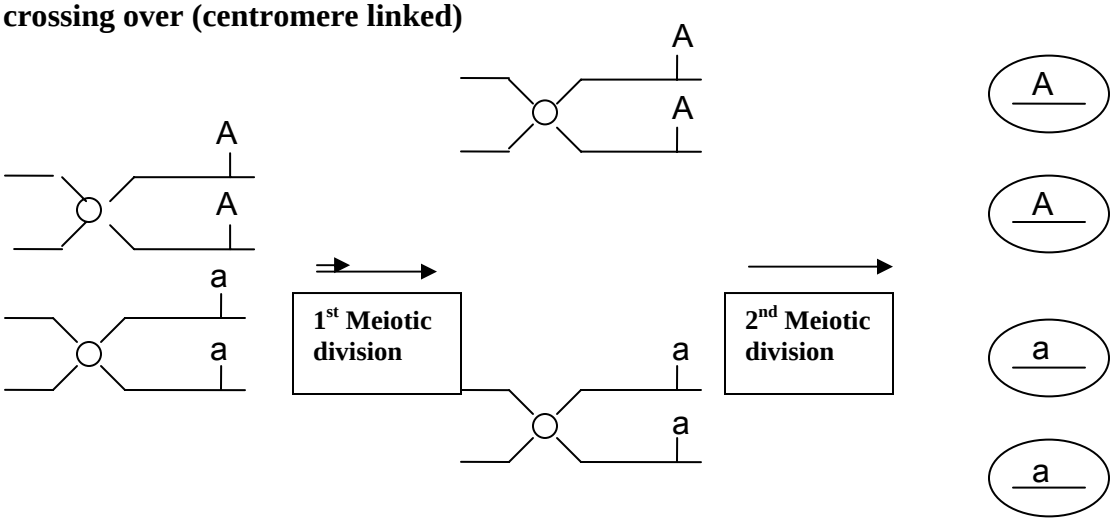
Thus, taking % SDS into consideration with a set of genes, a fairly accurate map in relation to centromere can be prepared. If for example, the diploid zygote contains *TRP1*/*trp1*(a centromere linked marker, approximately 1 cM from its centromere), the % of SDS is equal to the % of T asci with an error of less than 1%. This is so because *trp1* segregates along with the centromere, in majority of the cases at the first meiotic division. The percentage of SDS can also be determined with other centromere –linked genes that may not be as close as *trp1* and a relative map location can be worked out.

Gene complementation

Complementation tests are often carried out simultaneously to determine if the trait being followed is in the same gene or two different genes. For instance, two haploid strains (α and *a*) both being *leu* auxotroph, can be mated. If the resulting diploid is prototropic (it can grow on minimal medium) the functions of each mutant gene are said to be complemented (*leu1* / *LEU1 LEU2* / *leu2*) and hence the mutations are in two different genes. Such a phenomenon is also known as *intergenic complementation.* If however, the mutations are in the same gene, the gene

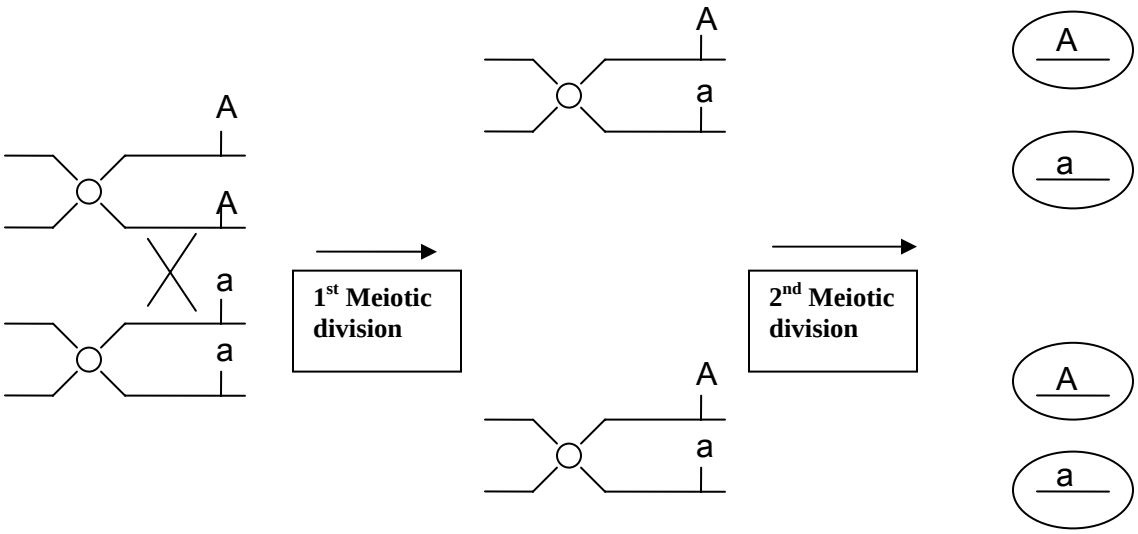
function cannot be complemented in diploid (*leu1 / leu2*) and it will fail to grow on minimal medium.

A. No crossing over (centromere linked)



(Products after first meiotic division,.segregation of alleles)

B. Crossing over (not linked to centromere)



(Products after first meiotic division, no segregation of alleles)

Fig. 9: Mapping of genes linked to centromere. (A) For a gene located close to the centromere, segregation will occur at the first meiotic division. (B) For a gene located some distance away, segregation will occur at the second meiotic division

Many proteins, on the other hand, can be constituted in different domains or functions. These two domains need not be a part of the same polypeptide chain to work or give final activity to the protein. The classic example is that of *Escherichia coli* β-galactosidase complementation, often used in selection of a recombinant molecule. Here, mutations in *lac Z* at the N-terminus of the two peptides (alpha and the omega peptides) are both products of two *lac Z* mutations, but complement each other to construct a fully functional protein (*α*-complementation), by way of intragenic complementation. In a case, where the protein function is due to more than one poly-peptide or a gene actually codes for two polypeptides, mutations in different parts of the gene could generate altered phenotypes which when complemented can result in original phenotype. Thus, complementation tests allow one to conclude whether two mutations are in different genes or are at different locations in the same gene.

Features of extra chromosomal inheritance

S. cerevisiae contains a circular plasmid (2μ) of 6318 bp. The plasmid encodes four proteins involved in plasmid maintenance and confers no particular phenotype on the cell. The cir0 strains lacking the plasmid have no observable phenotype. However, the chromosomal mutation nib1 causes reduced growth in cir+ strains due to abnormally high copy number of this plasmid. It is located within the nucleus and packaged into nucleosomes. It is maintained at high (~70) copy number due to 'Hip-Hop mechanism.' Its segregation from mother to daughter cells during mitosis is random unlike the chromosomal segregation. If the copy number drops to a low level, the rate of replication goes up to bring up the copy number. The origin of replication of this plasmid has been useful in constructing numerous shuttle vectors (YEp vectors) that can be maintained in yeast as well as in E. coli.

The mitochondrial DNA also is an important element contributing to extra chromosomal inheritance in yeast although the genome is compact and carries a limited number of genes. In all, about 15 genes (subunits of cytochrome b, cytochrome c oxidase, ATPase, one ribosomal protein, tRNAs, rRNA's) are present on the mitochondrial DNA. Mutations or deletions in genes responsible for respiration lead to the 'petite' phenotype, as these cells appear small when grown aerobically on glucose. If a petite yeast cell is mated with a wild type yeast cell, the diploid will contain both types of mitochondria. Due to poor doubling of the mutant mitochondria, these lose out in subsequent generations. The products of meiosis will inherit the wild type or normal mitochondria. The 'petite' cells resulting from mutations in chromosomally encoded respiratory proteins (cytochromosome c oxidase, F1 ATPase etc.) will show typical segregation, characteristic of the chromosomal gene.

Yeasts have a number of other DNA elements which are analogous to bacterial elements. These are mobile elements or retrotransposons such as Ty element, that transposes via an RNA intermediate. During transposition the cells accumulate virus-like particles which are not released outside of the cell but contain RNA, dsDNA reverse transcriptase and protease. The reverse transcribed cDNA copy of the RNA inserts into the genome and may cause mutation. Double-stranded RNA viruses have also been found in yeasts and constitute about 0.1 % of total nucleic acid. These are placed in three families namely L-A, L-BC, and M. The M double-stranded RNA encodes a killer toxin and L-A codes for the major coat protein and other components required for viral replication and maintenance of M. Both M and L-A are packaged

separately with the common capsid protein coded for by L-A resulting in formation of virus like particles. The viral particles accumulate inside the cell and are infectious only if the cells fuse with each other (i.e. during mating). L-B and L-C (collectively called as L-BC) are similar to L-A, have an RNA-dependent RNA polymerase activity and are present in intracellular particles. In addition to the double stranded RNA, the yeast also contains a 20S circular single stranded RNA encoding an RNA-dependent RNA polymerase that acts as an independent replicon. This is also inherited as a non-Mendelian genetic element.

Yeast genomics

One of the major impacts on classical gene expression studies has been that of knowledge of the genome and microarray technology. The genome sequence of over 100 organisms, including those of yeast, has been completed and the correct annotation (assignment of open reading frames with the possible function) of these has lead to fabrication of chips containing immobilized probes of every possible open reading frame of the yeast genome. Affymatrix chips are available that have nearly 3000 unique oligonucleotides representative of each gene, immobilized on a chip. Two sets of cells, cultivated under different experimental conditions (wild type vs mutant vegetative vs. sporulative) are chosen. Fluorescent total cDNA are prepared (each set with a dye that fluoresces at a given wave length) in two different PCR reactions. These reaction products are mixed, denatured to get single stranded DNA, which is next hybridized to oligo arrays. In this manner, differential expression profiles can be obtained for a pair of conditions or a pair of cells. The images are processed so that genes with identical expressions for the two sets are ignored while up-regulated and down-regulated genes are highlighted. Such analyses have been performed for a large number of cellular events and allow a deeper insight into biochemical networks operating within the cell. The linkages, hitherto not possible, of various biochemical reactions as well as identifying the role of previously unknown genes, has been possible using this technology.

Suggested Readings

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