JOINT SESSION — YEAST CELL BIOLOGY, MOLECULAR GENETICS OF FILAMENTOUS FUNGI, PLANT GENETICS

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Gene Regulation in Lower Eukaryotes

1550 THE MOLECULAR GENETICS OF FUNGI, Gerald R. Fink, Whitehead Institute/MIT, Cambridge, MA 02142

The techniques that have been developed for the study of gene function in yeast should be applicable to a wide variety of biological problems in diverse organisms. We have used these techniques to study the expression of both single copy genes like HIS4 and multi-copy genes like the Ty elements. Analysis of the HIS4 gene has identified both cis and trans-acting elements required for the expression of that gene. Most remarkable are the short, repeated, cis-acting elements: Two types are required to maintain the basal level of expression and a third is required for regulation through a global control, the general control of amino acid biosynthesis. The redundancy of genes like the Ty element (transposon yeast) makes the application of molecular genetic tricks considerably more difficult. We have been able to devise several strategies that circumvent these problems and provide answers to fundamental questions about the Ty elements:

Do the Ty elements transpose via a DNA or RNA intermediate?
What Ty structures are required in cis for transposition?
What Ty genes are required in trans for transposition?
What non-element functions are required for transposition?
Is the Ty element conserved during transposition?

1551 DETERMINATION OF YEAST CELL TYPE BY THREE REGULATORY ACTIVITIES (α1, α2 AND a1-α2) ENCODED BY MAT. Ira Herskowitz, Alexander Johnson, Stan Fields, Mike Hall & Kathy Wilson Dept of Biochemistry & Biophysics UCSF San Francisco CA 94143

Cell type in the yeast Saccharomyces cerevisiae is determined by alleles of the mating type locus, MATa and MATα (reviewed in 1; see also 2, 3). Cells with MATa have the a mating type, and cells with MATα have the α mating type. a and α cells mate efficiently with each other to form the third cell type, the a/α diploid cell.

MATα codes for two regulatory proteins:
--α1 **is a POSITIVE REGULATOR** of expression of a set of α-specific genes (αsg).
α1 is required for synthesis of RNA from the STE3 and MFα1 genes.
--α2 **is a NEGATIVE REGULATOR** of expression of a set of a-specific genes (asg).
α2 blocks synthesis of RNA from the STE2, STE6, and BAR1 genes.
MATa codes for one regulatory protein, a1, which has no known function in an a cell.
In an a/α cell, however:
--a1-α2 **is a NEGATIVE REGULATOR** of expression of a set of haploid-specific genes (hsg).
a1-α2 blocks synthesis of RNA from the MATα1, HO, and STE5 genes, as well as from the Tyl element.

Recent studies have added the following information.

--α1 is not sufficient for turning on α-specific genes such as MFα1 and STE3: two additional genes, STE12 and STE17, are also required (4).
--α2 protein (the negative regulator of a-specific genes such as STE6) binds to STE6 DNA upstream of the transcript start point (5).
--α2 binds to the upstream region of HO, a gene that is negatively regulated by a1-α2 (5).

1. Herskowitz, I. (1983) Determination of yeast cell type. Symp. Soc. Devel. Biol. 41:65-75 (Liss Inc.) 2. Wilson, K.L. & I. Herskowitz (1984) Molec. Cell. Biol., in press 3. Jensen, R., G.F. Sprague, Jr. & I. Herskowitz (1983) PNAS 80:3035-3039 4. S. Fields et al., in preparation 5. A. Johnson et al., in preparation

Gene Evolution and Action

1552 DISPERSED MULTIPLE-COPY GENES: WHAT KEEPS THEM HONEST? Robert L. Metzenberg, Eric U. Selker, Ewa Morzycka-Wroblewska, Judith Stevens, Dept. of Physiol. Chem., University of Wisconsin, Madison WI 53706.

The existence of multiple copies of certain genes in eucaryotes poses questions about how their sequences are kept identical in the face of mutation. Theoretically, a high degree of redundancy would make it difficult or impossible to eliminate new mutations by natural selection. However, in many cases, a high degree of uniformity is, in fact, maintained. One way in which this could be done is by cyclic expansion and contraction of the number of copies. If the repeated genes are tandemly arranged, expansion-contraction can occur by unequal crossingover. Expansion and contraction by transposition can accomplish the same end. The alternative to expansion-contraction is a correction mechanism in which the number of copies of the genes do not change, but information from one is used to "convert" another. This could involve contact between chromosomes, as in classical conversion, or such mechanisms as reverse-transcription of RNA coded from one gene or incorporation of the information into another gene.

In Neurospora crassa, there are about 200 tandemly-arranged genes coding for the three largest rRNA species and about 100 genes coding for 5S rRNA. The 5S genes are not tandemly repeated. We have therefore investigated the possibility of concerted evolution in this dispersed family. Chromosome mapping of over 20 cloned genes, using restriction fragment length polymorphisms, has shown that the genes are widely scattered. Unequal crossingover can therefore be excluded. A majority of these genes code for a 5S RNA form we call α, that other coding sequences, β, γ, etc. also exist, which differ from α and from one another in as many as 25 nucleotides out of 120. Surprisingly, the flanking sequences are all very different, even those containing identical coding sequences. If classical gene conversion is occurring between non-allelic coding sequences, its extent is exactly limited to the transcribed region. To test the possibility of transposition and deletion, we cloned DNA segments corresponding to those flanking regions from a strain distantly related to our laboratory strain. In each case, the clones were found to contain a 5S gene in the same position as in the canonical laboratory strain, indicating that the 5S gene is not being inserted or deleted with high frequency. The possibility that genes are "converted" by an RNA-mediated process remains attractive, but has not been tested.

While the major transcript in vivo is α, there are at least six species of 5S RNA in cells. Most, if not all, of these are present in ribosomes, and presumably function in protein synthesis. The number, sequence, and proportion of 5S RNA types is highly conserved in different species within the genus Neurospora and even in members of related genera. Hence the plurality of 5S RNA types is probably not an accident, but serves some function.

1553 PLANT TRANSPOSABLE ELEMENTS : A FACTOR IN GENOME EVOLUTION ? Peter Starlinger, Institut für Genetik, Universität zu Köln, D-5000 Köln 41, Federal Republic of Germany

Transposable elements of many organisms consist of a particular DNA sequence each. Most of them terminate in inverted repeats and nearly always they cause a short sequence duplication at their sites of insertion. Plant transposable elements make no exception, but they have additional properties that will be described mainly for the maize element Ac, mentioning similarities to other elements where possible. Among these observations are:

1) the finding of element families derived from the original element by internal deletions,
2) aberrant elements sharing with the main element terminal sequences of variable length, but carrying additional sequences. Some of these seem to be generated from the main element by a sequence-shuffling mechanism,
3) the formation of composite structures of increasing complexity from the basic elements,
4) chromosome aberrations of different length caused by the Ac element and/or its derivatives. A model for these events will be discussed,
5) retention of the duplication of the host DNA at the insertion site upon excision of the element. The retained duplication is usually mutated.