news and views

Intervening sequences in the mitochondrial genome

from Giorgio Bernardi

SINCE its foundation by Boris Ephrussi 30 years ago, mitochondrial genetics has made great advances and yet this subject has remained an area of interest to only a small number of laboratories. Whatever the explanation for this, the situation is not likely to last much longer since exciting results recently obtained in one particular system, the mitochondrial genome of yeast, should stimulate very wide interest.

The mitochondrial genome of *Saccharomyces cerevisiae* comprises about 100 identical, circular, supercoiled DNA molecules per diploid cell. Each genome unit is about 50 million in molecular weight, a value five times greater than that of the mitochondrial genome of organisms as far apart in terms of evolution as *Chlamydomonas* and man, but smaller (by a factor of almost two) than that of higher plants. Each genome unit is known to encode two ribosomal RNAs, at least 30 tRNAs, and nine proteins: three of the seven subunits of cytochrome *c* oxidase, one of the seven subunits of the *bcI* complex, three of the ten subunits of the ATPase complex, and one protein, *varI*, of unknown function. The other mitochondrial proteins are specified by nuclear genes, a fact which emphasises the very close cooperation between nuclear and mitochondrial genes in mitochondrial biogenesis and function.

The fivefold difference in unit size between the mitochondrial genomes of yeast and, say, man, is difficult to explain in terms of coding potential since it is unlikely that the former codes for so many more gene products than the latter. Put in a different way, the discrepancy between the unit size of the mitochondrial genome of yeast, about 50 million, and the amount of DNA

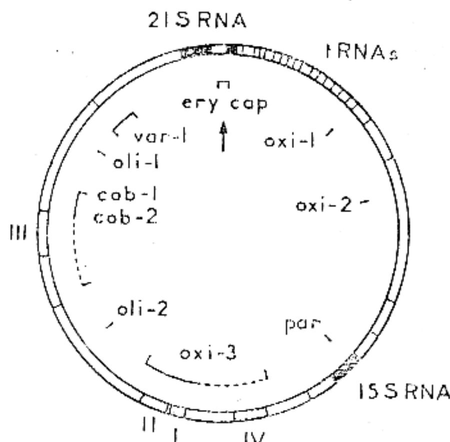

Fig. 1 The mitochondrial genome of *S. cerevisiae* modified from Borst & Grivell *Cell* (in the press). The location of the genes for 21S and 15S RNAs is indicated. Thin, radial lines indicate the position of tRNA genes. Roman numerals indicate genome segments absent in *S. carlsbergensis*. The 21S RNA insert is represented by the dotted region splitting the 21S RNA gene. The approximate location of a number of genetic markers as well as the *cob* loci referred to in the text is indicated inside the ring. The arrow indicates the origin of the map which corresponds to a *SalI* site.

required to encode its known gene products, about 10 million, raises the same problem as the so-called 'c-value paradox' of the nuclear genome of eukaryotes, namely, what is the role of the 'excess DNA'? An answer to this problem is emerging from two different experimental approaches.

A direct molecular approach revealed, some years ago, that half of the mitochondrial genome of yeast consists of AT spacers (GC<5%) formed by short, repeated dAT : dAT and dA : dT sequences. The other half, presumed to correspond to the structural genes and to their regulatory elements, is extremely heterogeneous in base composition (since it ranges from 21% GC for ribosomal genes, to about 35% GC for tRNA genes, to 65% GC for GC-rich segments representing 10% of the genome) and contains 60–70 clusters of the sequences CCGG and GGCC[1] which are palindromic and homologous in sequence[2,3], as originally postulated by Prunell and Bernardi. By cutting these clusters with the restriction enzyme *Hae*III, the genome can be split into 60–70 fragments, most of which seem to be formed by a GC-rich region, a gene (or gene segment, see below), and a spacer. Interestingly enough, the *Hae*III fragments vary in length and (to some extent) in number in different strains, two factors which are responsible for a variation of ±5 in the size of the corresponding genomes[4].

The functions of the spacers, of the (CCGG, GGCC) clusters and of the GC-rich segments are still a matter of conjecture. The presence in them of homologous or quasi-homologous sequences appears, however, to be responsible for the high frequency of recombination phenomena which characterise the mitochondrial genome of yeast. Unequal crossing-over events seem to account for the changes in length of *Hae*III fragments, which accompany genome evolution[4] and genome recombination in sexual crosses[5] as well as for the excision of genome segments leading to the defective genomes found in spontaneous 'petite' mutants[6].

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The starting point for the genetic approach was the discovery of the so-called mit⁻ mutants in which mitochondrial functions are impaired by point mutations or small deletions affecting the mitochondrial genes coding for the subunits of respiratory enzymes or of ATPase. Mapping work in several laboratories has revealed that both the *cob* (or *box*) region, coding for apocytochrome *b* (the mitochondrial subunit of the *bc1* complex), and the *oxi-3* locus (which codes for subunit I of cytochrome *c* oxidase) correspond to DNA segments several times larger than expected (see Fig. 1). More recent work[8] indicates that non-coding regions and regulatory sequences are present within the *cob* region. The data of Slonimski *et al.*, for instance, indicate that mutants lacking functional cytochrome *b* map in seven discrete clusters dispersed over 6,000–10,000 base pairs. Mutations at non-adjacent loci 4/5, 1 and 6 fall into a single complementation group and produce abnormal apocytochrome *b*. On the other hand, mutations at loci 2, 3 and 7 complement mutations at loci 4/5, 1 and 6, complement each other, and present a disturbed synthesis of both subunit I of cytochrome *c* oxidase and of cytochrome *b*. Most of these pleiotropic mutants are temperature-sensitive and accumulate multiple 'new' mitochondrial translation products. The genetic and biochemical work just mentioned is further supported by electron microscopic work[9] on hybrids between the 18S mRNA transcribed from the *cob* region and restriction fragments from the same region; this shows that the gene for cytochrome *b* consists of at least five coding regions separated by four non-coding regions which are between 600 and 2,000 base pairs long.

At least one other mitochondrial gene, the gene for the 21S RNA, is also split, in addition to the two already mentioned. Electron microscopy of 21S RNA with a restriction fragment containing this gene, show that this contains a 1000 base pair insertion which is closely correlated with the ω^+ allele, a polarity locus affecting the transmission of neighbouring genetic markers in crosses[10,11]. Strains having the ω^- allele lack the insertion, which is always transmitted in $\omega^+ \times \omega^-$ crosses to the ω^+ progeny. Very interestingly, a large intervening sequence[12] containing tRNA genes[13,14] has been found in the gene for the 24S RNA of *Neurospora* mitochondria.

An important question raised by these findings concerns the relationship of intervening sequences with the sequence elements (spacers and GC-rich segments) discussed above. More specifically, it may be wondered whether some of the homologous sequences present in those elements are not used in the RNA splicing. While current biochemical and genetic work will certainly lead to an answer to this question, it is noteworthy that the large regions of strain-dependent genome variability are correlated with the presence of long dA:dT sequences and map at the *cob* and *oxi-3* regions (Fig. 1). Finally, it should be noted that while the presence of split genes further weakens the hypothesis of the prokaryotic origin of mitochondria, its main interest undoubtedly resides in the fact that intervening sequences have now an easy genetic handle which should allow us to gain a better understanding of these puzzling genome elements. □

Enzyme defects and immune dysfunction

from Fred S. Rosen

GENETIC deficiency of two enzymes in the purine degradation pathway, adenosine deaminase (ADA) or nucleoside phosphorylase (NP), results in fatal immunodeficiency disease. E. Giblett (University of Washington, Seattle) discovered ADA deficiency serendipitously while examining erythrocytes for informative enzyme polymorphisms from a child with severe combined immunodeficiency (SCID), a profound deficiency of T and B lymphocytes. She subsequently discovered NP deficiency in a child with severe T cell deficiency. These are the only genetically determined immunodeficiencies that have a known biochemical basis. Although ADA and NP are distributed in all mammalian tissues, only the lymphoid system is affected by the deficiencies.

A meeting was held recently at the Ciba Foundation* to discuss the biochemical consequences of ADA and NP deficiency, particularly as to how they affect the ontogeny of lymphocytes.

Both ADA and NP deficiency are inherited as autosomal recessive phenomena. ADA has been assigned to chromosome 20 and NP to chromosome 14. ADA deficiency results in a more profound immunodeficiency than does NP deficiency. Of all infants affected with the autosomal recessive form of SCID, 35 to 50% have ADA deficiency. NP deficiency is known in only six kindred. Affected children in these six kindred have a severe A cell deficiency, normal immunoglobulins, increased antibody responses, autoantibodies, and poor *in vitro* responses to mitogenic stimuli (A. Ammann, University of California, San Francisco). By contrast, ADA deficiencies have a profound defect of both T and B cells and no *in vitro* or *in vivo* immunological function. Although the erythrocytes are completely deficient in ADA, a mutant enzyme has been found in fibroblasts and lymphocytes in low amounts (approximately 15% of normal). This has enabled the prenatal diagnosis of this disease in amnion fibroblasts (R. Hirschhorn, New York University, New York).

It was found that NP deficients accumulate 2′-deoxyguanosine triphosphate (dGTP) in erythrocytes, and lymphocytes whereas ADA deficients have abnormally high levels of 2′-deoxyadenosine triphosphate (dATP) in these cells (A. Cohen & D. W. Martin, University of California, San Francisco). D. A. Carson (Scripps, La Jolla) showed that preferential T cell trapping of deoxyadenosine and deoxyguanosine and subsequent accumulation of dATP and dGTP is due to the high kinase content of T cell lines and their relative poverty of 5′-nucleotidase. L. Thelander (Karolinska Institute, Stockholm) obtained calf thymus ribonucleotide reductase in high purity. The allosteric regulation of this enzyme requires 1×10^{-3} M ATP as a positive effector, and is readily inhibited by dATP and to a lesser extent by dGTP and dTTP. dCTP has no inhibitory effect. In fact, dCDP can rescue murine mutant S49 lymphoma cells that have ADA and/or NP deficiency. N. M. Kredich (Duke University, Durham) offered a very reasonable explanation for the greater severity of ADA deficiency in that adenosine accumulation inhibits the hydrolase of S-adenosylhomocysteine. This substrate accumulates and clogs the methyl donor activity from S-adenosylmethionine. Thus DNA synthesis in ADA⁻ lymphocytes is obstructed at the ribonucleotide reductase step and DNA methylation is inadequate.

Establishment of lymphoid chimaerism with transplants of histoidentical marrow has been the most satisfactory therapy for ADA deficiency. Lacking a bone marrow donor for an ADA deficient infant, S. H. Polmar (Case Western Reserve, Cleveland) used frozen human erythrocyte transfusions as a source of exogenous ADA. This succeeded in reducing dATP levels in lymphocytes and inducing normal immune function. This procedure was extended with further success to an NP deficient child (B. J. M. Zegers, Wilhemina Kinderziekenhuis, Utrecht). W. N. Kelley (University of Michigan, Ann Arbor) has obtained both ADA

*Enzyme Defects and Immunity Dysfunction, held 7–9 November. The Proceedings of the conference and the discussion will be published in their entirety for the Ciba Foundation by Excerpta Medica, Amsterdam.

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