Genes for bacterial and mitochondrial ATP synthase

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The structures of the ATP synthases in bacteria and mitochondria are very similar (for reviews see Senior, 1979; Fillingame, 1981; Racker, 1981; Walker et al., 1982). They are membrane-bound enzymes composed of two distinct domains: a domain called $F_o$ which is buried in the membrane and attached to it a domain known as $F_i$ which lies outside the membrane (see Fig. 1). $F_i$ is composed of five different polypeptides, $\alpha$, $\beta$, $\gamma$, $\delta$ and $\varepsilon$, and bacterial $F_o$ is made up from three proteins, $a$, $b$ and $c$. Mitochondrial $F_o$ may be more complex, but contains counterparts to the bacterial subunits. The catalytic sites of the enzyme are in $F_i$ and $F_o$ has a proton channel through which the membrane proton-potential gradient generated by electron transport is coupled to phosphorylation of ADP.

In mammals, all of the subunits of the enzyme are encoded in nuclear genes except those for subunit $a$ (also called ATPase-6) and for a smaller protein that appears to be important for assembly of the enzyme complex (Macreadie et al., 1983). These two proteins are encoded in mitochondrial DNA (Anderson et al., 1981, 1982). A similar arrangement is found in yeast (but not Neurospora), except that the gene for the $c$ subunit (also called the proteolipid or DCCD-binding protein) is also in mitochondrial DNA (Macino & Tzagoloff, 1980). The genes for the Escherichia coli enzyme are grouped in an operon, the unc operon (Walker et al., 1983). As illustrated in Fig. 1 this arrangement is related to the structure of the enzyme in so far as genes for $F_o$ components $a$, $c$ and $b$ are clustered at the promoter proximal end and are followed by genes for $F_i$ subunits in the order $\delta: \alpha: \gamma: \beta: \varepsilon$. It is noteworthy that the order $\beta: \varepsilon$ is also found in maize and spinach chloroplast DNA (Krebers et al., 1982; Kurawski et al., 1982). Clustering of genes with related functions is a feature of the genetic maps of coliphages such as T4 and lambda (Wood & King, 1979). For example, in lambda, morphogenetic genes for head proteins form a cluster adjacent to a cluster for tail genes (Katsura, 1983); in T4, genes for base plate, head and tail also form clusters. It has been suggested that these gene clusters might reflect the evolutionary origin of present-day assembly genes by tandem duplication and divergence of ancestral assembly genes (Casjens & Hendrix, 1974). In addition, or alternatively, clustering could reflect the selective advantage of minimizing recombination between genes for proteins that interact structurally, so as to decrease the probability of non-viable hybrids in interstrain matings (King & Laemmli, 1973; Casjens & Hendrix, 1974; Kikuchi & King, 1974). In lambda the similarity between the order

Fig. 1. Arrangement of subunits in the E. coli ATP-synthase complex and the order of genes in the E. coli unc operon

The subunit stoichiometry is probably $\alpha_3\beta_2\gamma_1\delta_1\varepsilon_1a_1b_2c_{10-12}$. kb, kilobases.
of action of gene products during assembly (J, L, K, H, G, M, V, U, Z) and the order of genes in the lambda genome (J, K, L, M, H, G, V, U, Z) is a good example in support of this idea. A third possibility is that proteins that interact with each other are synthesized near to each other in the cell (Katsura, 1983). A fourth explanation for gene order in the lambda morphogenetic locus was advanced by Parkinson (Katsura, 1983). A fourth explanation for gene order in the lambda genome was subsequently discounted (Katsura, 1983). Similar considerations to these may apply to the unc operon. For example, evolutionary relationships between genes are apparent, a being related to β (Walker et al., 1982) and related sequences are present in δ and ε in β and ε (Walker et al., 1984).

In order to see if the gene order in the unc operon of *E. coli* is maintained in other micro-organisms, genes for ATP-synthase subunits have been cloned and sequenced from a photosynthetic non-sulphur bacterium *Rhodopseudomonas blasticus*. For this purpose parts of the *E. coli* α and β genes were used as probes for hybridization with a library of the *Rhodopseudomonas* genome constructed by Dr. J. Sulston. These experiments have shown that the genes for F1 subunits form an operon (see Fig. 2) which does not contain genes for F0 subunits. As yet the locations of F0 genes are unknown. The order of the F genes is the same as that for the homologous *E. coli* genes. However, an additional potential gene, χ, is found between the γ and β genes. This DNA encodes a hydrophilic protein of unknown function. It is not related to the *E. coli* unc1 gene product, a membrane protein of unknown function (Gay & Walker, 1981; Gay, 1983).

**Potassium transport in Escherichia coli:** genetic and biochemical characterization of the K+-transporting ATPase*

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In *Escherichia coli*, K+ is accumulated by at least two transport systems: the constitutive Trk and the repressible high-affinity Kdp system. When the K+ concentration in the medium falls below 0.2 mM, the kdpABC gene cluster is switched on. Transcription is initiated by the kdpD gene product (Rhoads & Epstein, 1978). The latter is a membrane protein that is thought to respond to a decrease in turgor pressure by adopting a conformation favourable for interaction with the kdp promoter (Laimins et al., 1981). The gene products encoded by the kdpA, kdpB and kdpC genes have been identified as intrinsic proteins of the cytoplasmic membrane with relative molecular masses of 47000, 90000 and 22000 respectively (Laimins et al., 1978).

Studies performed on both intact cells and isolated membranes have shown that the expression of these proteins is correlated with the appearance of a K+-stimulated ATPase activity (Epstein et al., 1978; Wieczorek & Altendorf, 1979). The Kdp complex has been purified in an ATPase-active form using Aminoxid WS 35 as a detergent. As expected, ATPase activity of the isolated enzyme was stimulated by K+. However, not all ions that were transported through the native complex stimulated ATPase activity of the purified enzyme, and vice versa. Re-stimulated ATPase activity, although this ion was not accepted as a transport substrate in vivo. Conversely, T1 did not stimulate ATPase activity although it was accumulated through the Kdp transport system in vivo (Damper et al., 1979). Although Na+ is probably not a substrate for the

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