Kdp transport system, this ion at low concentrations stimulated and at high concentrations inhibited ATPase activity. Furthermore, Kdp ATPase activity was also inhibited by ADP, N-ethylmaleimide and vanadate, but not by ouabain or chlorhexydrin.

Isolation of the Kdp ATPase with [γ-32P]ATP revealed that in analogy to eukaryotic ion pumps a phosphorylated intermediate was formed during ATP hydrolysis. The participation of this phosphoprotein in a phosphorylation–dephosphorylation cycle was confirmed by pulse-chase experiments. Addition of non-radioactive ATP led to a fast turnover of the 32P-labelled intermediate. Both the pH-lability profile of the intermediate and its sensitivity towards hydroxylamine suggest the involvement of an acyl-phosphate bond, which has tentatively been assigned to an aspartyl residue on subunit B. K⁺ induced a partial turnover of the phosphorylated intermediate; Rb⁺, Na⁺ and Li⁺ were less effective. Both phosphorylation and dephosphorylation were strictly dependent upon Mg²⁺. Vanadate and N-ethylmaleimide blocked dephosphorylation.

Genetic analysis of K₄ mutants of the Kdp system suggested that the kdpA gene product is involved in K⁺ binding; as yet direct evidence for its involvement in the K⁺-translocation step is lacking.

Biochemical studies have revealed that ATPase activity is associated with the KdpB subunit. The function of the KdpC protein still remains obscure. Since this subunit is absolutely necessary for transport activity it may play a role in the proper assembly of the enzyme complex (Epstein & Davies, 1970).

Proteolytic digestion and radioactive-surface-labelling studies with diazobenzenesulphonic acid on right-side-out and inverted vesicles indicated that the bulk of the KdpB protein is protruding from both sides of the membrane. By contrast, the main portions of the KdpA and KdpC proteins appear to be embedded in the membrane. Starting from the purified enzyme complex the three subunits of the Kdp ATPase have been isolated by sodium dodecyl sulphate-column chromatography. So far, the amino acid composition and the N-terminal sequence of subunit C have been determined. Furthermore, the kdpABC operon has been cloned and the DNA sequences of the three subunits have partially been established.

Isolation and manipulation of genes coding for energy-transducing enzymes from Neurospora crassa and Escherichia coli

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Energy-transducing enzymes of the mitochondrial inner membrane, including ATP synthase, the bc complex and cytochrome oxidase, have complicated subunit compositions. The cloning and isolation of the coding sequences (mRNAs or genes) will produce the possibility of gathering information on the amino acid sequences of these subunits. Moreover, the isolated nucleic acids can potentially be manipulated to allow study of the functional properties of these proteins. Some of these subunits are coded on mitochondrial DNA, and their corresponding genes have already been analysed and sequenced due to the relatively small size of this genome (Slonimskii et al., 1982). Most of the subunits are, however, encoded in the nucleus, translated in the cytosol (some of them as larger precursors) and subsequently imported into mitochondria. Owing to the large size of the nuclear genome, cloning and isolation of these genes is more difficult.

The ATP synthase occurs in remarkably similar form in mitochondria, chloroplasts and bacteria. Therefore the essential properties of the subunits can be studied by a comparison of the primary structure from phylogenetically distant organisms. In Escherichia coli all eight subunit genes are organized as one operon (the ATP operon), and they have been cloned on a phage (Miki et al., 1978; Hansen & von Meyenburg, 1980). In addition, mutants are available where single or several subunit genes are deleted. The E. coli system is therefore the system of choice for the expression of single subunits of the ATP synthase, and for the study of the resultant phenotypes (Friedl et al., 1983).

Isolation of cloned mRNA from Neurospora crassa

It can be estimated that in the lower eukaryote Neurospora crassa the mRNAs encoding subunits of mitochondrial energy-transducing complexes occur at frequencies of approx. 0.5–5 per 1000 (Viebrock et al., 1982). In order to identify a cloned mRNA of this abundance, in an ordered cDNA bank, methods were adapted for the cell-free translation of hybrid-selected mRNA. The techniques were optimized so that in one experiment about 1000 clones could be analysed. Furthermore, the cell-free translation products of the hybrid-selected mRNAs could be precipitated with several specific antibodies, either simultaneously or in separate, successive incubations. Several rounds of hybridization could be performed, since cDNA plasmids had been covalently coupled to diazo benzoyloxymethyl-paper.

Up to now, cDNAs have been identified which code for the proteolipid subunit and the β subunit of the ATP synthase, for the ATP/ADP translocator, and for the iron-sulphur protein and two of the smaller subunits of the bc complex. cDNAs corresponding to most of each of the mRNAs of the proteolipid and of the ATP synthase, for the respective operon have been isolated by sodium dodecyl sulphate-column chromatography. So far, the amino acid composition and the N-terminal sequence of subunit C have been determined. Furthermore, the kdpABC operon has been cloned and the DNA sequences of the three subunits have partially been established.

Characteristics of cloned proteins and genes from Neurospora crassa

The ATP synthase proteolipid subunit, an extremely hydrophobic protein of 81 amino acid residues, is synthesized as a precursor with a presequence of 66 amino acids. The presequence is very polar. It contains 12 basic residues and no acidic ones. The presequence apparently renders the proteolipid soluble, so that it can be imported into mitochondria post-translationally.

The nuclear gene contains two introns, one in the middle of the presequence, and one just before the last amino acid

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Molecular and genetic aspects of the fumarate reductase of Escherichia coli

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Fumarate reductase is an anaerobically inducible, membrane-bound flavoprotein complex which effects the terminal step of an anaerobic electron-transport chain, employing fumarate as the ultimate electron acceptor. Electrons obtained from abundant substrates such as \( \alpha \)-glycerophosphate, lactate, formate, hydrogen or NADH are transferred via specific dehydrogenases, \( b \)-type cytochromes and menaquinone to fumarate reductase which then reduces fumarate to succinate. This process, common among both facultative and obligate anaerobic bacteria, generates a proton motive force which can be used for the synthesis of ATP or for active transport (Kröger, 1978).

The best-understood fumarate reductase is that from *Escherichia coli* as it has been extensively characterized at both molecular genetic and biochemical levels. Initial studies with a specific group of mutants unable to grow anaerobically on non-fermentable substrates with fumarate as terminal electron acceptor identified the structural genes (*frd*) for the enzyme at 94 min on the *E. coli* linkage map and showed the reductase to be distinct from the tricarboxylic acid cycle enzyme, succinate dehydrogenase, which also interconverts fumarate and succinate (Hirsch et al., 1963; Spencer & Guest, 1973; Bachmann & Low, 1980). By means of complementation of defined *frd* lesions, specialized transducing phages bearing the genes encoding fumarate reductase were isolated from \( \lambda \) gene banks (Cole & Guest, 1978, 1980a). These phages all contained a 4.9 \( \times \) 10^3 base-pair HindIII fragment which was later shown to be present in the Clarke and Carbon gene bank (Edlund et al., 1979; Guest, 1981). Determination of the entire nucleotide sequence of this fragment revealed the *frd* genes to be arranged in an operon (Fig. 1a) consisting of a regulatory region followed by four cistrons, *frdA,B,C,D*, coding for components of the enzyme, and a promoter (Cole, 1982; Cole et al., 1982; Grundström & Jaurin, 1982). A striking feature of the operon is its remarkable sequence economy: the *frdA* cistron partially overlaps *frdB* whereas the most distal cistron *frdD* contains the promoter region for the next gene, *ampC*, within its coding sequence (Fig. 1a). One consequence of the latter arrangement is that the *frd* transcriptional terminator also serves to attenuate the growth-rate-dependent expression of the *ampC* gene (Grundström & Jaurin, 1982). Further sequence economy is found within the intercistronic regions as in two cases the translational stop codons have been incorporated into the ribosome-binding sites of the following cistron.

**Fig. 1.** Organization of the *frd* genes of *E. coli* and a model for the arrangement of fumarate reductase in the membrane

(a) The precise locations of the *frdA*, *B*, *C*, *D* and *ampC* genes, transcriptional terminators and the approximate location of the promoters are shown together with the *M* of the proteins they encode (K, kilodaltons). An unidentified gene is denoted *gx*. The relevant restriction sites and the scale in 10^3 \( \times \) base-pairs are indicated. (b) A schematic model for the arrangement of fumarate reductase in the membrane. Note that the precise association of the four subunits (A, B, C, D) is not known.

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