Mitochondrial Adenosine Triphosphatase of the Fission Yeast *Schizosaccharomyces pombe* 972h⁻

CHANGES IN INHIBITOR SENSITIVITIES DURING THE CELL CYCLE INDICATE SIMILARITIES AND DIFFERENCES IN BINDING SITES

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1. We used 11 different inhibitors of energy conservation as inhibitors of ATPase (adenosine triphosphatase) in extracts of *Schizosaccharomyces pombe* obtained from cells at different stages of the cell cycle. 2. All the inhibitors showed cell-cycle-dependent variations in their I_{50} values (μg of inhibitor/mg of protein giving 50% inhibition of inhibitor-sensitive ATPase at pH 8.6). 3. From the sensitivity profiles through the cell cycle it was concluded that: (a) oligomycin, venturicidin, triethyltin sulphate and dibutylchloromethyltin chloride all act at closely associated site(s); (b) *NN'*-dicyclohexylcarbodi-imide and leucinostatin both act at a similar site, which is, however, distinct from that at which other inhibitors of the membrane factor (F_0) act. 4. The variations in I_{50} values for efraeptin closely followed changes in specific activity of ATPase, as would be expected for an inhibitor acting at catalytic sites; these fluctuations were different from those for aurovertin, Dio-9, 4-chloro-7-nitrobenzofurazan, quercetin and spegazzinine, all of which show different sensitivity profiles from one another. 5. Anomalous stepwise inhibitor-titration curves were obtained for spegazzinine, *NN'*-dicyclohexylcarbodi-imide, dibutylchloromethyltin chloride and leucinostatin. 6. Possible explanations are proposed for the discontinuous expression of inhibitor-binding sites during the cell cycle.

The mitochondrial ATP synthetase complex (oligomycin-sensitive ATPase*) of yeasts, like that from ox heart, has been shown to be comprised of three functional components: F_1 -ATPase (composed of five different polypeptide subunits), the oligomycin-sensitivity-conferring peptide and the membrane factor [F_0 , consisting of four different subunits (Kagawa & Racker, 1966; Schatz *et al.*, 1967; Tzagoloff *et al.*, 1973; Goffeau *et al.*, 1972, 1973)]. A naturally occurring inhibitor of F_1 -ATPase has been isolated and characterized from both mammalian and yeast systems (Pullman & Monroy, 1963; Brooks & Senior, 1971; Satre *et al.*, 1975; Landry & Goffeau, 1975); this peptide may be regarded as an eleventh easily dissociated subunit of the complex.

The presence of a natural inhibitor in catabolite-de-repressed *Schizosaccharomyces pombe* is correlated, not only with decreased ATPase activity (Landry & Goffeau, 1975), but also with increased sensitivity to inhibitors of energy conservation (Lloyd & Edwards, 1976). Thus, when the natural

inhibitor is present, sensitivity to those inhibitors that act on F_1 -ATPase shows a 2-fold increase, whereas sensitivity to inhibitors that bind to the membrane factor (F_0) shows a much larger increase. These differences between the properties of mitochondrial ATPase of catabolite-repressed and -de-repressed yeasts provide confirmation of the relative positions of inhibitor-binding sites within the enzyme complex.

In a study of the time-course of development of mitochondrial ATPase during the cell cycle of *S. pombe* in synchronous cultures, it was shown that the periodic expression of enzyme activity is accompanied by a 4-fold variation in oligomycin-sensitivity (Edwards & Lloyd, 1977). This observation was confirmed by titration of ATPase with oligomycin in extracts of cells from different stages of the cell cycle obtained by the technique of cell-cycle fractionation.

In the present study we have extended this approach to 11 other inhibitors of mitochondrial ATPase: venturicidin, triethyltin sulphate, dibutylchloromethyltin chloride, *NN'*-dicyclohexylcarbodi-imide and leucinostatin, all of which bind to the membrane factor, F_0 ; Dio-9; aurovertin, efraeptin, Nbf-Cl, quercetin and spegazzinine [all of which

* Abbreviations: ATPase, adenosine triphosphatase (EC 3.6.1.3); Nbf-Cl, 4-chloro-7-nitrobenzofurazan (previously named 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole and abbreviated Nbd-Cl).

interact with F_1 -ATPase (Lloyd & Edwards, 1976)]. As well as suggesting the occurrence of a sequential expression of different inhibitor sites during the assembly and integration of mitochondrial ATPase in the normal process of cell growth, the results give more detailed information on the relative sites of interaction of different inhibitors with the enzyme complex. Thus time-resolution of the assembly and integration processes *in vivo* provides information that cannot be obtained from classical fractionation regimes or by the strategy of reconstitution.

Experimental

Maintenance, growth and harvesting of the organisms

S. pombe 972h⁻ was maintained on 3% (w/v) malt-extract agar and grown with forced aeration in minimal medium containing 1% (w/v) glucose as described previously (Poole *et al.*, 1973; Poole & Lloyd, 1973). Organisms were counted as described previously (Edwards & Lloyd, 1977).

Cultures were harvested in the phase of glucose de-repression (at a cell population of between 3.9×10^7 and 6.4×10^7 organisms/ml at 4°C) by continuous-flow centrifugation in an MSE continuous-action rotor (flow rate, 1 litre/min; rotor speed, 8000 rev./min). Yeasts were sedimented from concentrated suspensions thus produced, by acceleration to 6000 rev./min, followed by immediate deceleration in the 6×250 ml rotor of an MSE 18 centrifuge. Organisms were resuspended in water and resedimented.

Cell-cycle fractionation by zonal centrifugation

Cell-cycle fractionation was by rate-zonal centrifugation on a linear unbuffered sucrose gradient (total vol. 590 ml) in an HSA zonal rotor running in an MSE 18 centrifuge as described previously (Poole & Lloyd, 1973). Sedimentation of the organisms was at 1500 rev./min for 5 min. Fractions (20 ml) were collected and sucrose concentrations were determined by using a refractometer; they were then centrifuged at 9000 rev./min (8000g, r_{av} 7.6 cm) for 10 min in the 8×50 ml rotor of an MSE centrifuge to sediment the cells. These were resuspended in 6.5 ml of 50 mM-Tris/ H_2SO_4 buffer, pH 8.6, and sedimented by acceleration to 6000 rev./min followed by immediate deceleration. Each fraction was finally made up to exactly 6.5 ml with buffer. Cell numbers and size distributions of cell populations in each fraction were immediately determined by using a Coulter Counter model Z_B fitted with a P64 Channelyzer. Median cell volumes were calculated from integrated cell-size-distribution plots; mean cell volumes were evaluated by balancing cardboard cutouts of these plots on a knife-edge held at 90° to the x-axis. A portion of

each fraction (1.5 ml) was stored at -18°C before assays for total protein.

Preparation of cell-free extracts

Preparation of cell-free extracts was as described previously (Lloyd & Edwards, 1976).

ATPase assays

ATPase was assayed by pH 8.6 by the proton-release method as described previously (Lloyd & Edwards, 1976).

Protein

Protein was measured in cell-free extracts by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Total cell protein was determined by the method of Herbert *et al.* (1971), with the same standard.

Chemicals

Sources of most chemicals have been given previously (Lloyd & Edwards, 1976). Dibutyl-chloromethyltin chloride was from Dr. D. E. Griffiths and Mr. K. Cain, Department of Molecular Sciences, University of Warwick, Coventry, U.K. Leucinstatin was from Dr. R. Hamill of Lilly Research Laboratories, Minneapolis, IN, U.S.A.

Results

Cell-cycle analysis by zonal centrifugation: changes in ATPase activity and sensitivity to venturicidin, triethyltin and Dio-9

Rate-zonal centrifugation of an exponentially growing population of *S. pombe* separates classes of cells on a size basis (Poole & Lloyd, 1973). Cell volume increases continuously over the first three-quarters of the cell cycle and then remains constant during the formation of the cell plate (Mitchison, 1957). Some separation of cells at different stages of the constant-volume phase may also occur on the gradients, because of density changes (Mitchison & Carter, 1975). Fig. 1 shows typical size-distribution plots obtained on analysis of individual fractions from a cell-cycle-fractionation experiment. Values for median and mean cell volumes calculated from Fig. 1 are shown in Fig. 2(a), together with cell numbers and total protein recovered in each fraction of the gradient. ATPase activities of cell-free extracts show a complex pattern of expression with four maxima (Fig. 2b). Early in the cell cycle virtually all the ATPase detected was sensitive to venturicidin, triethyltin and Dio-9. Over the third quarter of the

Fig. 1. Size distributions of cell populations after zonal centrifugation of a glucose-de-repressed exponentially growing culture of *S. pombe*

A suspension (50 ml) containing 15 g wet wt. of cells in 5% (w/v) sucrose was prepared after harvesting a culture containing 3.9×10^7 cells/ml and loaded on to a linear sucrose density gradient in an MSE HSA zonal rotor. Centrifugation was at 1500 rev./min for 5 min (7200 g-min at the sample zone, $\int_0^t \omega^2 dt = 11000 \text{ rad}^2/\text{s}$). Size distributions of cell populations in fractions were determined by using a Coulter Counter model Z_B fitted with a P-64 Channelyzer (64 channels). Settings were as follows: I×A, 1; lower threshold, 10; upper threshold, off; matching resistance, 20 k Ω ; gain setting, 5; edit switch, on. The orifice diameter was 70 μm . Channels with most organisms contained 2048 counts. Numbers refer to fraction numbers in the experiment shown in Fig. 2: (a) fractions 1 (—), 3 (---) and 6 (····); (b) fractions 8 (—), 11 (---) and 13 (····).

cell cycle inhibition of ATPase was less complete; venturicidin was more effective than Dio-9, and triethyltin gave least inhibition. In all cases the inhibitor-sensitive activities closely followed the total ATPase activity in their patterns of expression.

Measurements of values of I_{50} (μg of inhibitor/ μg of protein giving 50% inhibition of inhibitor-sensitive ATPase at pH 8.6) for the three inhibitors indicate remarkable fluctuations through the cell cycle (Fig. 3); thus sensitivity to venturicidin shows a 16-fold variation; the corresponding ranges observed for triethyltin and Dio-9 were 5-fold and 2-fold respectively. The overall patterns of inhibitor sensitivities were similar for venturicidin and triethyltin and were reminiscent of the oscillations in oligomycin-sensitivity previously observed both in synchronous cultures and on cell-cycle fractionation of catabolite-de-repressed organisms (Edwards & Lloyd, 1977). Maximum inhibitor resistances approximately corresponded with maxima in enzyme activities for both these inhibitors. Dio-9 resistance showed two maxima, but was quite different from the other inhibitors in the first half of the cell cycle.

Changes in sensitivity to aurovertin, efrapeptin, Nbf-Cl and quercetin during the cell cycle

Fig. 4(a) shows the distribution of cell numbers, median cell volumes and mean cell volumes after sedimentation of an exponentially growing population of catabolite-de-repressed *S. pombe* through a linear

sucrose gradient. Assays for ATPase activity in cell-free extracts prepared from cells harvested from successive fractions again reveal the presence of four maxima (Fig. 4b). I_{50} values for efrapeptin (Fig. 4c) show that the sensitivity of the enzyme complex to this inhibitor is directly related to the specific activity of the enzyme, i.e. enzyme activity and I_{50} values are maximal at the same stages in the cell cycle. Profiles of sensitivity to three other F_1 -ATPase inhibitors, aurovertin (Fig. 4c), Nbf-Cl (Fig. 4d) and quercetin (Fig. 4d), are all different from one other and from that for efrapeptin, although responses to efrapeptin and Nbf-Cl show some similarities over the second half of the cell cycle. Maximum resistance to aurovertin occurs twice per cycle, as does that for quercetin, although the timing of the phases of maximal resistance in the middle one-third of the cycle is quite distinctive for each of these two compounds. Inhibitor-titration curves for these four inhibitors of F_1 -ATPase are shown in Fig. 5; I_{50} values range from 0.004 to 0.062 for efrapeptin (15-fold), from 0.5 to 9.4 (19-fold) for aurovertin, from 1.0 to 17.0 for Nbf-Cl (17-fold) and from 1.0 to 20.3 (20-fold) for quercetin.

Changes in sensitivity to spegazzinine, dibutylchloromethyltin and leucinostatin

Results from another cell-cycle-fractionation experiment are shown in Fig. 6. Again measurements of median and mean cell volumes indicate that a

Fig. 2. Analysis of the cell cycle by rate-zonal centrifugation of cells from a glucose-de-repressed exponentially growing culture of *S. pombe*

Culture and centrifugation conditions are described in the legend to Fig. 1. (a) Mean cell volumes ($\blacktriangle$), median cell volume ($\triangle$), sucrose density gradient ($\bullet$) and distribution of cell numbers ($\circ$); (b) total ATPase ($\bullet$) and ATPase activities sensitive to venturicidin ($\circ$), (c) triethyltin ($\triangle$) and Dio-9 ($\blacktriangle$).

satisfactory spread of cell sizes through the linear sucrose gradient (Fig. 6a) and hence the fractions obtained may be related to timings on a cell-cycle 'map'. The profile of sensitivity to spegazzinine, a dihydroindole alkaloid, which is known to react with purified F_1 -ATPase (Roveri & Vallejos, 1974), is clearly quite different from that obtained for NN' -dicyclohexylcarbodi-imide (Figs. 6c and 6d) an inhibitor that binds to the membrane factor (F_0) (Cattell *et al.*, 1971). On the other hand sensitivity profiles for NN' -dicyclohexylcarbodi-imide and leucinostatin show striking similarities; these are different from the variations in I_{50} values for dibutylchloromethyltin between different fractions (Fig. 6d).

Fig. 3. Variations in values of I_{50} for Dio-9, venturicidin and triethyltin through the cell cycle of *S. pombe*. Culture and centrifugation conditions are described in the legend to Fig. 1. I_{50} values (μg of inhibitor/mg of protein) for Dio-9 ($\triangle$), venturicidin ($\bullet$) and triethyltin ($\triangle$).

Some representative inhibitor-titration curves obtained in this experiment are presented in Fig. 7. Remarkable stepwise inhibition curves were obtained for most (but not all) fractions with these four inhibitors, in contrast with the classical patterns for inhibitor titrations observed with all other inhibitors. I_{50} values range from 0.6 to 22.0 (36-fold) for spegazzinine, from 0.04 to 7.4 for NN' -dicyclohexylcarbodi-imide (185-fold), from 0.1 to 3.35 for leucinostatin (33.5-fold) and from 0.0026 to 0.046 for dibutylchloromethyltin chloride (17.7-fold).

Discussion

We have previously shown that different inhibitors of mitochondrial ATPase may be classified in two groups on the basis of changes in values for their I_{50} during the transition from catabolite repression to de-repression during the growth of the fission yeast *S. pombe* with glucose (Lloyd & Edwards, 1976). With the exception of Dio-9 these two groups correspond to (a) those which are known to interact with ATPase in submitochondrial particles (or with purified oligomycin-sensitive ATPase), but not with purified F_1 -ATPase (i.e. those which bind to the membrane factor, F_0), and (b) those which react with F_1 -ATPase itself.

Fig. 4. Analysis of the cell cycle by rate-zonal centrifugation of cells from a glucose-de-repressed exponentially growing culture of *S. pombe*: variations in values of I_{50} for efrapeptin, aurovertin, Nbf-Cl and quercetin

A suspension (50ml) containing 21g wet wt. of cells was prepared after harvesting cells at a population of 5.4×10^7 cells/ml and loaded on to a sucrose density gradient in an MSE HSA zonal rotor. Centrifugation was at 1500 rev./min for 5 min ($7200g$ -min at the sample zone, $\int_0^5 \omega^2 dt = 11000 \text{ rad}^2/\text{s}$). (a) Mean cell volumes ($\circ$), median cell volumes ($\blacktriangle$), sucrose density gradient ($\blacksquare$) and distribution of cell numbers ($\bullet$); (b) specific activity of ATPase ($\bullet$); (c) sensitivities of ATPase [I_{50} values (μg of inhibitor/mg of protein giving 50% inhibition of the inhibitor-sensitive ATPase)] towards efrapeptin ($\bullet$) and aurovertin ($\circ$); (d) I_{50} values for Nbf-Cl ($\triangle$) and quercetin ($\blacktriangle$).

Temporal resolution of the expression of ATPase activity through the cell cycle revealed that changes in activity were correlated with changes in sensitivity to inhibition by oligomycin over a 4-fold range of values for I_{50} (Edwards & Lloyd, 1977). Comparison

of profiles of inhibitor-sensitivities through the cell cycle provides a method for further elucidation of similarities and differences between other antibiotics and reagents in inhibitor-enzyme interactions. On the basis of this criterion we can sub-classify

Fig. 5. Effect of inhibitors on ATPase activities in extracts prepared from *S. pombe* at different stages of the cell cycle

Culture and centrifugation conditions are given in the legend to Fig. 4. Sequential additions of small volumes (1 μ l) of solutions of inhibitors in dimethylformamide were made to ATPase incubation mixtures at pH 8.6. After correction for the inhibitory effect of the solvent (10% at 20 μ l of solvent/2 ml total vol.), inhibition (%) was expressed as a function of inhibitor titres. (a) Titration curves produced on adding efrapectin to fractions 1 (●), 3 (○), 5 (Δ), 7 (▲), 9 (■) and 12 (□); (b) titration curves produced on adding aurovertin to fractions 1 (●), 3 (○), 7 (▲), 12 (Δ), 13 (□) and 14 (■); (c) titration curves produced on adding Nbf-Cl to fractions 2 (●), 7 (○), 10 (▲), 11 (Δ), 13 (□) and 14 (■); (d) titration curves produced on adding quercetin to fractions 2 (●), 5 (○), 11 (Δ), 12 (▲), 13 (■) and 14 (□).

inhibitors of F_0 into two groups: (a) oligomycin, venturicidin and the two organotin derivatives, triethyltin and dibutylchloromethyltin chloride;

(b) NN' -dicyclohexylcarbodi-imide and leucinostatin. Lardy *et al.* (1975) have also suggested that the binding sites for leucinostatin are different from those for oligomycin. Genetic studies have established that two independent cistrons located on mitochondrial DNA are involved in venturicidin resistance; one of these is common to an oligomycin-sensitivity site and the other is common to a site that determines a triethyltin-sensitivity site (Lancashire & Griffiths, 1975; Griffiths *et al.*, 1975). Dibutylchloromethyltin chloride, a site-directed affinity-label derivative of triethyltin, reacts with six binding sites on oligomycin-sensitive ATPase (Griffiths & Hyams, 1977); the functional group of subunit 9, which is modified, is lipoic acid (Griffiths *et al.*, 1977). It has often been assumed that NN' -dicyclo-hexylcarbodi-imide and oligomycin act at similar sites on F_0 (Senior, 1973), but the preparation of a purified ATPase, which is sensitive to oligomycin, but not to NN' -dicyclohexylcarbodi-imide (Serrano *et al.*, 1976), makes this unlikely. Thus results obtained by careful analyses of the time-dependent modifications of the ATP synthetase complex through the cell cycle confirm and extend observations made by several other independent approaches.

The present data indicate that sensitivity to efrapectin, an antibiotic that is thought to react at or close to the catalytic site of F_1 -ATPase (Lardy *et al.*, 1975), closely follows changes in enzyme activity. Aurovertin, an antibiotic that also reacts with F_1 -ATPase (Lardy *et al.*, 1964; Robertson *et al.*, 1967; Slater & Ter Welle, 1969), but at a site other than the catalytic site (Chang & Penefsky, 1973; Lardy *et al.*, 1975), shows a sensitivity profile through the cell cycle that is completely different from that for efrapectin. Nbf-Cl, a chemical modifying agent, which reacts with a single tyrosine residue likely to be on the β -subunit of F_1 -ATPase from ox heart (Ferguson *et al.*, 1975a,b), gives a sensitivity profile that is similar to that obtained for efrapectin only over the second half of the cell cycle. This suggests that the action of these two inhibitors is related, but not identical. It is also notable that efrapectin is a much more effective inhibitor (i.e. has a lower I_{50} value) than Nbf-Cl. The other inhibitors of F_1 -ATPase, Dio-9 (Guillory, 1964), quercetin (Lang & Racker, 1974) and spegazzanine (Roveri & Vallejos, 1974), each show distinctive sensitivity profiles, which are also different from those of efrapectin, aurovertin and Nbf-Cl. This strongly suggests that their binding sites are also different, although the small fluctuations in Dio-9 sensitivity makes for difficult comparison with other inhibitors. Scheme 1 shows the components of the ATP synthetase complex and possible location of binding sites.

For most of the inhibitors studied, conventional patterns of inhibition were observed (i.e. the extent

Fig. 6. Analysis of the cell cycle by rate-zonal centrifugation of cells from a glucose-de-repressed exponentially growing culture of *S. pombe*: variations in values of I_{50} for spegazzinine, NN' -dicyclohexylcarbodi-imide, leucinostatin and dibutylchloromethyltin chloride

A suspension (50ml) containing 20g wet wt. of cells was prepared after harvesting cells at a population of 6.4×10^7 cells/ml and loaded on to a linear sucrose gradient in an MSE HSA zonal rotor. Centrifugation was at 1000 rev./min for 5.8 min (5400g-min at the sample zone, $\int_0^t \omega dt = 8000 \text{ rad}^2/\text{s}$). (a) Mean cell volumes (Δ), median cell volumes ($\circ$) sucrose density gradient ($\bullet$) and distribution of cell numbers ($\circ$); (b) specific activity of ATPase ($\bullet$); (c) sensitivities of ATPase [I_{50} values (μg of inhibitor/mg of protein giving 50% inhibition of the inhibitor-sensitive ATPase)] towards NN' -dicyclohexylcarbodi-imide ($\circ$) and leucinostatin ($\bullet$); (d) I_{50} values for spegazzinine ($\circ$) and dibutylchloromethyltin chloride ($\bullet$).

of inhibition increased with increasing amounts of inhibitors up to a plateau value). However, anomalous inhibition curves were obtained in certain fractions with dibutylchloromethyltin chloride, NN' -dicyclohexylcarbodi-imide, leucinostatin and speg-

azzinine. Further studies are required to understand this phenomenon, but possible explanations include intersubunit interactions following inhibitor binding or the redistribution of mobile components of the energy-conservation system [e.g. lipoic acid (Griffiths

Fig. 7. Effect of inhibitors on ATPase activities in extracts prepared from *S. pombe* at different stages of the cell cycle. Culture and centrifugation conditions are given in the legend to Fig. 6. Sequential additions of small volumes ($1\ \mu\text{l}$) of solutions of inhibitors in dimethylformamide were made to ATPase incubation mixtures at pH 8.6. After correction for the inhibitory effect of the solvent (10% at $20\ \mu\text{l}$ of solvent/2 ml total volume), inhibition (%) was expressed as a function of inhibitor titres. (a) Titration curves produced on adding spegazzinine to fractions 1 (●), 3 (○), 6 (▲), 8 (△), 10 (■) and 12 (□); (b) titration curves produced on adding *NN'*-dicyclohexylcarbodi-imide to fractions 2 (●), 3 (○), 5 (△), 7 (▲), 9 (■), 11 (▽) and 13 (□); (c) titration curves on adding dibutylchloromethyltin chloride to fractions 2 (○), 5 (□), 6 (△), 8 (▲), 12 (■) and 14 (●); (d) titration curves on adding leucinostatin to fractions 1 (●), 2 (○), 4 (▲), 6 (△) and 9 (□).

Scheme 1. *ATP synthetase complex and probable sites of action of inhibitors in S. pombe based on discontinuities in the temporal expression of inhibitor-binding sites*

et al., 1976)] within the complex. Substitution of dibutylchloromethyltin chloride-inhibited components by non-inhibited components from a pool in the inner mitochondrial membrane can lead to reversal of inhibition; stimulation of ATPase activity at high concentrations of *NN'*-dicyclohexylcarbodi-imide has also been observed (Landry & Goffeau, 1975).

A number of alternative explanations for fluctuations in inhibitor sensitivities through the cell cycle may be suggested: (a) periodic synthesis of those subunits bearing specific inhibitor-binding sites; (b) periodic binding of subunits from a pre-existing precursor pool; (c) periodic modification of existing membrane-bound ATPase (by restricted proteolysis, or changes in enzyme conformation at the level of tertiary or quaternary organization) so as to expose latent inhibitor-binding sites; (d) periodic modification of the membrane in the vicinity of the enzyme (change in fatty acid or sterol composition); (e) periodic synthesis of compounds that absorb or inactivate added inhibitors; (f) modulation of the ATP synthetase complex by binding of F₁ inhibitor peptide.

Further work should enable estimation to be made of the relative importance of these various parameters in the expression of properties of the mitochondrial ATPase. The experimental approach

adopted in this study provides information on the pathway and temporal organization of assembly, integration, and functional modulation of this enzyme complex *in vivo* during the growth and division of cells in the absence of metabolic disturbances.

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