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GLUCOSE EFFECT AND MITOCHONDRIOGENESIS IN YEAST[1]

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SUMMARY

The effect of addition of glucose to derepressing yeast cells on the electron transport chain components has been studied. Both the concentration and the time of addition were varied. The results show that the components made by mitochondria are extremely sensitive to glucose repression, whereas the cytoplasmically made counterparts (mitochondrially located proteins) are not.

Yeast cells grown in presence of glucose, not only rapidly lose their mitochondria but also are unable to synthesise new organelle (1). As soon as the glucose in the medium is exhausted, mitochondriogenesis is initiated and this derepression process is characterised by a stepwise appearance of various components (1,2). It was therefore of interest to study the effect of glucose on the derepressing cells with the expectation that this might lead to the identification of some specific glucose sensitive step. The results presented in this communication point out that products made by mitochondria are extremely sensitive to glucose repression.

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METHODS

A diploid strain, Saccharomyces cerevisiae 3095 was used in these studies. The growth conditions for bringing about the repression and derepression of mitochondria have been fully described earlier (3). Under these conditions, the cells remain in the fully repressed stage upto 2.5 h from the time of inoculation into the glucose containing medium and subsequently derepression is initiated. Mitochondria were obtained from the cells by grinding cells with a mixture of carborundum-celite (1:1 w/w) and suspended in 0.1M phosphate buffer (pH 7.4). The homogenate so obtained was subjected to differential centrifugation (3). Oxygen uptake was measured polarographically (3). Succinate dehydrogenase (4) from the homogenate, cytochrome oxidase (5) from mitochondria, ubiquinone (6), cardiolipin (7) and cytochromes a, b and c as pyridine hemochromes (8).

RESULTS

Under the experimental conditions, the yeast cells remained repressed upto 2.5 h, as seen from the oxygen uptake measurements and thereafter derepression starts and the oxygen uptake increases (Fig.1). Addition of glucose at 2.5 h prevented this derepression. Addition of even as low as 0.1% glucose to the medium was sufficient to prevent the rise in oxygen uptake but derepression starts early.

In a further series of experiments, addition of glucose (to 1% level) was made at 3 h, when the cells are derepressing and samples were taken after 30 min time intervals. Various components were assayed in homogenate, mitochondria and in whole cells. The results are shown in Table 1. Oxygen uptake capacity, levels of cardiolipin and cytochrome heme b decrease to the low repressed level. On the other hand, succinate dehydrogenase, ubiquinone, cytochrome hemes a and c, showed levels much higher than in the normally derepressing cells.

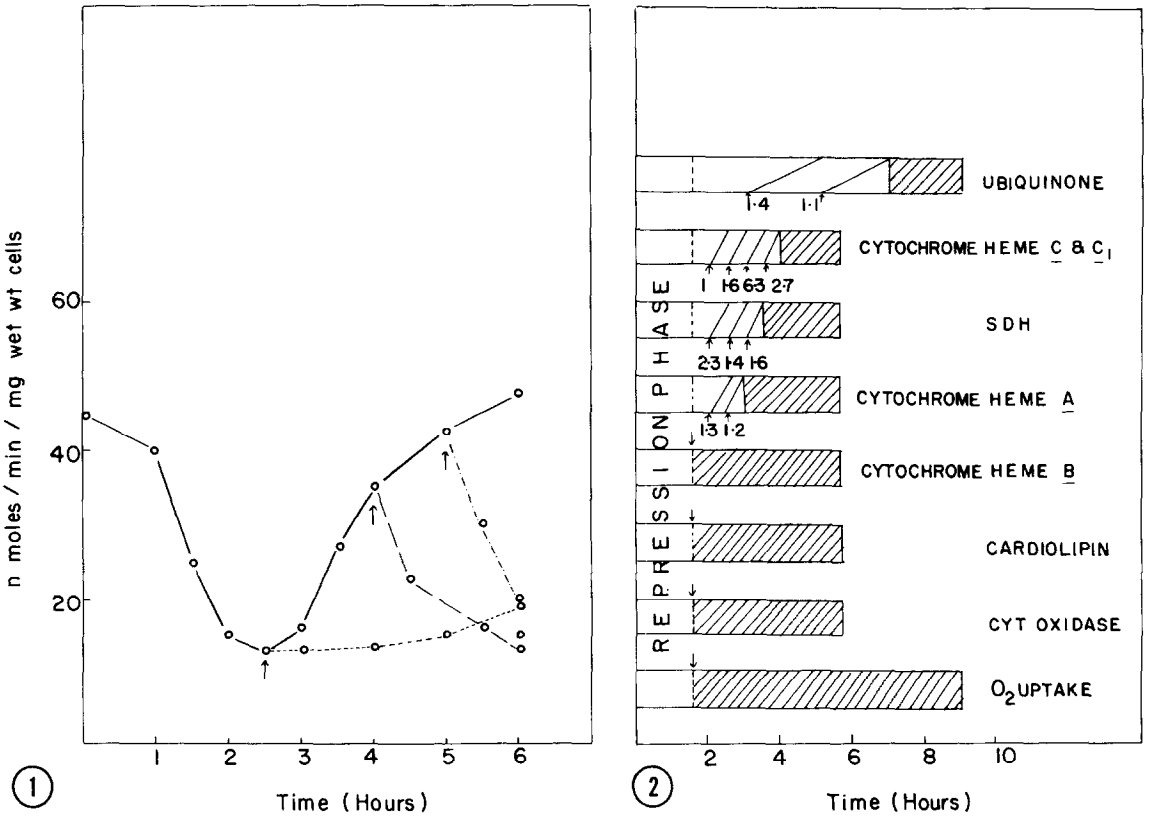

Fig.1 Glucose addition during derepression:

Glucose was added to a final concentration of 1% at different times of derepression and the oxygen uptake was measured at subsequent time periods

Fig.2 Summary of the response of various compounds to glucose addition:

Hatched area represents time periods where addition of glucose (1%) reduces the contents below the repressed levels. Open areas indicate the time periods where glucose addition increases the levels. The arrows indicate the time of glucose addition and time of harvest, while the figures represent the fold increase in the levels over the control derepressing cells.

In the next series of experiments, the addition of glucose was made at different times during derepression and the various components were analysed after 30 min as before

Table 1

Components		Activity at 2.5 h	Activity at 3.5 h	
			No glucose	Plus glucose
A	Oxygen uptake	12	17	11.5
B	Cytochrome oxidase	0.40	0.60	0.50
C	Cardiolipin	138	170	154
D	Cytochrome heme b	15	45	15
E	Cytochrome heme a	9	27	33
F	Cytochrome hemes c and c_1	3	9	14
G	Ubiquinone	10	22	30.5
H	Succinate dehydrogenase	1.4	4.0	5.4

The cells were grown in 1% glucose for a time period of 3 h. Glucose was added (1%) and various parameters were assayed

A	n moles/min/mg cells
B	μ moles cyt c oxidised/min/mg mit protein
C	ng phosphorous/mg mit protein
D,E & F	n moles/mg cell protein
G	μ moles/g cells
H	μ moles dye reduced/min/mg protein

(Fig.2). It was found that one group of components: cytochrome oxidase, cytochrome heme b and cardiolipin showed immediate sensitivity and their levels were brought down to the repressed levels irrespective of the time of addition.

The components succinate dehydrogenase, cytochrome hemes a and c and ubiquinone showed a different behaviour. Their levels showed an increase over the normal cells as a response to glucose addition for a certain period. Addition of glucose beyond this time, decreased the levels, and were brought down to those of the repressed cells. Further among the components studied, each one had a definite time when the glucose sensitivity was manifest. Response of cytochrome heme a levels to glucose was seen only after 60 min eventhough the activity of the enzyme cytochrome oxidase was affected immediately. The results are shown in Fig.2. Experiments were carried out to determine the lowest level of glucose needed to bring about this effect on the two enzymes cytochrome oxidase and succinate dehydrogenase. When only 0.1% glucose was added in the medium cytochrome oxidase activity was decreased by about 60% whereas SDH activity showed about 25% increase as compared to the control (data not shown).

DISCUSSION

The molecular mechanism by which glucose brings about a repression of mitochondriogenesis in yeast remains largely unknown. Glucose has been shown to enhance the activities of vacuolar hydrolytic enzymes and this has been ascribed as the cause of mitochondrial breakdown (9). But glucose also inhibits the formation of new mitochondria. The results presented in this communication, show that addition of glucose to derepressing yeast cells, brings about a differential effect on the levels of various components. Cytochrome

oxidase, cardiolipin and cytochrome heme b which are sensitive to glucose have been shown to require products synthesized on mitochondrial ribosomes either in part or as a whole (10,11,12). Whereas the other components which are not affected by addition of glucose, have their origin in the cytosol (12,13,14). We have recently shown (15) using immunoprecipitation techniques, that in presence of cyclohemide the mitochondrial subunits of cytochrome oxidase are synthesized and addition of glucose blocks this. Cyclic AMP reverses this action of glucose. Preliminary studies also show that addition of glucose delocalises the enzyme ATPase from its membrane bound state, a situation analogues to chloramphenicol addition. It is known that the binding of ATPase to the membrane needs mitochondrially made membrane factor (16). Several workers have shown that on inhibition of mitochondrial protein synthesis by chloramphenicol, the enzymes made in the cytoplasm e.g., succinate dehydrogenase, show enhanced levels (14,17). A similar result is obtained on treatment with glucose also.

Based on these results, we suggest that the primary effect of glucose is the inhibition of mitochondrial protein synthesis. At the moment we do not have any explanation, as to why the various cytoplasmically made products react differentially to the time of addition of glucose except that their turnover rates are involved.

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