Protein Synthesis and Amino Acid Pools in Irradiated Yeast Cells

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(Z. Naturforsch. **32 c**, 973–978 [1977]; received August 29, 1977)

Yeast, Protein Synthesis, Amino Acid Pools, Irradiation

Protein synthesis after UV- and X-irradiation was investigated in diploid yeast. The incorporation of radioactively labelled lysine and phenylalanine was measured 2.5 and 4 hours after exposure. By varying the specific activity the pool sizes could be estimated. At 2.5 hours there is some increase in pool sizes and a dose-dependent enhancement of protein synthesis. At 4 hours pools are again normal, but the increase of synthetic activity prevails.

Introduction

Relatively low doses (in terms of influence on colony forming ability) of X-rays or ultraviolet (UV)-irradiation cause severe alterations in cellular metabolism [1, 2]. It has been suggested that radiation-induced derepression of operons [3, 4] may be responsible for these effects which ultimately lead to cell killing. Protein synthesis plays obviously a key role in this respect. The protein content of irradiated cells is increased over control values and amino acid incorporation proceeds at an elevated rate [2]. The latter, however, must not necessarily reflect a higher synthesis rate because changes in pool size could lead to a change in intracellular specific activity of the precursor. This may be checked by varying the amount of unlabelled amino acids. Results of this type of investigation are reported in the present paper and analyzed by means of a simple mathematical model. It is found that some changes of pool size occur 2.5 hours after irradiation and that protein synthesis is increased in irradiated yeast cells.

Theoretical

Since the protein synthesizing machinery cannot distinguish between labelled and unlabelled precursors the specific activity of a newly formed macromolecule is proportional to the specific activity of the amino acids incorporated.

Because of intracellular pools, however, this is not identical with the specific activity added to the medium.

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Let P^* be the specific activity of the newly synthesized protein, a^* the amount of labelled precursor, a that of the unlabelled precursor added and a_0 the pool size. Then one may write for a given incubation period

$$P^* = s \cdot \frac{d\,a^*}{d\,a + a_0}. \qquad (1)$$

s is a proportionality factor related to synthesis rate and d a parameter relating intracellular and extracellular precursor concentration. Here it is assumed that there is linear dependence between both which is justified with low concentrations. Equation (1) may be rearranged

$$1/P^* = \frac{1}{s} \cdot \frac{a}{a^*} + \frac{a_0}{s \cdot d\,a^*}. \qquad (2)$$

If one varies a with constant a^* a straight line relationship is expected between $1/P^*$ and a where the inverse of the slope indicates synthetic activity. Extrapolation to the abscissa $(1/P^* \to 0)$ yields the value of $-(a_0/d)$.

Whereas synthesis rate may be directly estimated the pool size a_0 appears only in combination with the "permeability factor" d (see Discussion).

Experimental

Wild type diploid yeast, strain 211 (originally obtained from W. Laskowski) was grown at 30 °C in Wickerham medium [5] to stationary phase. Cells were harvested by centrifugation, washed and resuspended in water for irradiation (3×10^7 cells per ml for UV, 10^8 cells per ml for X-rays). Exposure took place in open Petri dishes after which the cells were inoculated into fresh medium at a concentration of 10^7 cells/ml and incubated for various times at 30 °C in a shaking water bath.

Incorporation:

2.5 hours and 4 hours after irradiation 1 μCi/ml of [^{3}H]phenylalanine (specific activity 1 Ci/mmol) or [^{3}H]lysine (specific activity 19 Ci/mmol) and varying amounts of nonradioactive precursor were added. After 15 min the incorporation was stopped by 20% trichloroacetic acid (TCA) to bring the mixture to 10% TCA. Samples were washed with 5% TCA, lyophilized, boiled for 15 min in 5% TCA and collected on a membrane filter. After drying the radioactivity was measured in a Philips liquid scintillation counter. In parallel cell numbers were counted microscopically and the protein content determined by the method of Lowry *et al.* [6].

Irradiation source were a low pressure mercury lamp (OSRAM HNS 12) and a Philips 100 kVp X-ray tube. The dose rates were 2.2 Wm^{-2} and 48 krad/min, respectively, determined by chemical actinometry [7] in the case of UV and ferrous sulphate dosimetry with X-rays.

Results

Fig. 1 shows plots of lysine incorporation rates according to Eqn. (2) in irradiated cells after 2.5 hours of incubation. The respective data for phenylalanine are given in Fig. 2. It may be seen that – with one exception – there is very little change in the apparent pool size (a_0/d) of phenylalanine, whereas there is some increase for lysine in all irradiated cells. Only with 264 J m^{-2} UV-exposure this parameter is significantly increased. The slope is also steeper indicating a reduced synthesis rate compared to the control, both for lysine and phenylalanine. In all other cases the synthesis activity is increased in irradiated cells. This is even more obvious after 4 hours (Figs 3 – 6). There are no significant changes in apparent pool sizes (a_0/d_0) but always reduced slopes in the exposed samples. The relative synthesis as measured by the incorporation of the two precursors determined from the slopes of the respective graphs is summarized in Table I. For comparison colony forming abilities are also given.

Table I. Relative incorporation rate in irradiated cells after 2.5 and 4 h incubation.

X-ray dose [krad]	2.5 h		4 h		Colony forming ability [%]
	Rel. phe-inc. [%]	Rel. lys-inc. [%]	Rel. phe-inc. [%]	Rel. lys-inc. [%]	
30	236	252	206	178	75
60	—	207	247	96	42
90	150	—	206	107	8
UV-dose [Jm^{-2}]					
66	153	377	188	179	80
132	—	237	275	246	52
198	—	—	188	179	31
264	89	66	—	—	19

Discussion

It appears obvious from the given data that protein synthesis is mostly increased in irradiated cells, both at 2.5 and 4 hours after UV- or X-ray exposure. This is not due to pool changes because they are – if altered at all – increased over control values. This would result in reduced incorporation rates, thus underestimating the true synthesis rate if only measurements with one particular specific activity were made. The elevated synthesis rate is more pronounced for lower doses; with higher doses there is again a reduction. There are distinct differences between the two amino acids as affected by UV after 2.5 hours and by X-rays after 4 hours. This may indicate that synthesis rate is not uniformly increased for all cellular proteins, but that there is also a change in protein composition. This question will be pursued in future investigations.

It is not possible to measure pool sizes unequivocally by our method, if knowledge of the "permeability parameter" d is lacking. It seems to be safe, however, to draw the conclusion that there is no difference to the controls after 4 hours because it is very unlikely that a_0 and d are changed exactly in such a way that the alterations would cancel each other. After 2.5 hours there may be either a change in a_0 or in d or even in both. We feel, however, that a decrease in d is not very probable because it is known that irradiated cells increase their surface and become more permeable to a variety of substances. The data might, therefore, indicate a radiation-induced enlargement of the precursor pool.

The interpretation of our experiments may be obscured by amino acid leakage into the medium which is well known to occour in irradiated cells. The doses are, however, much higher than used here: Swenson and Dott [8] observed significant amino acid leakage in yeast after an UV-dose of 2142 J m^{-2} and only very little after 714 J m^{-2},

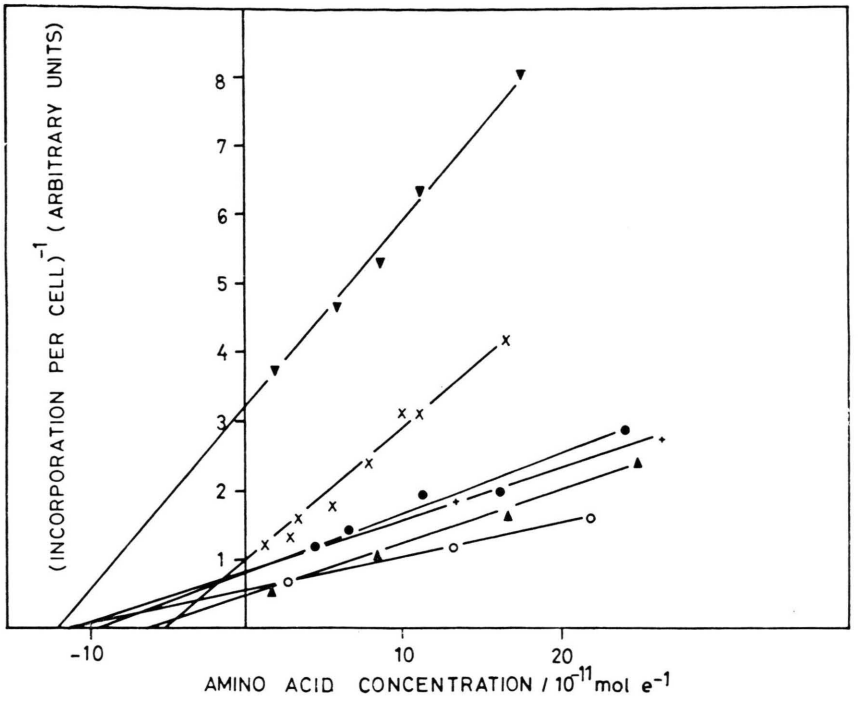

Fig. 1.
Incorporation of [^{3}H]phenylalanine 2.5 hours after irradiation.
×: controls;
○: 66 Jm^{-2} UV;
△: 264 Jm^{-2} UV;
▼: 30 krad X-rays;
●: 90 krad X-rays.

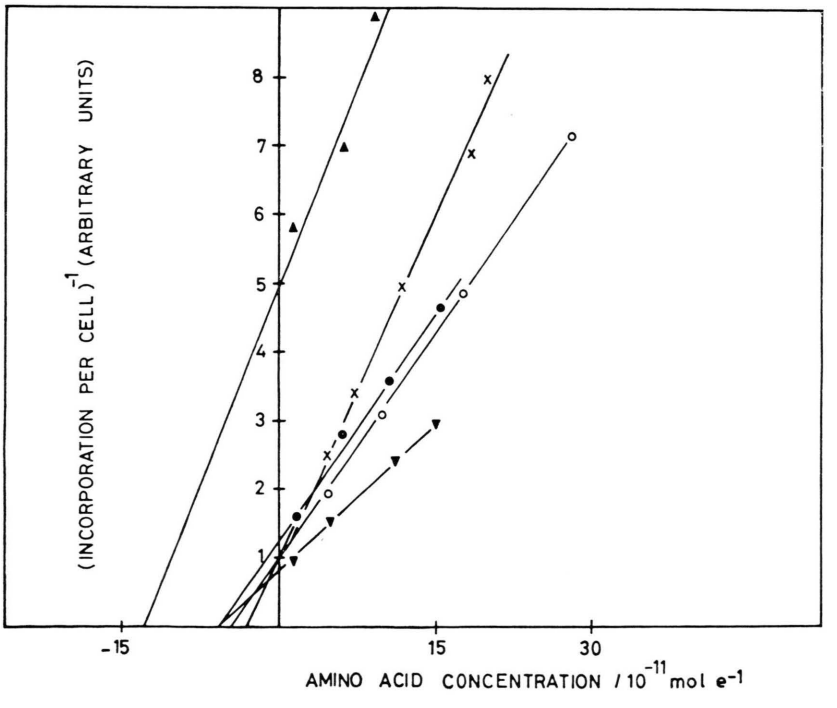

Fig. 2.
Incorporation of [^{3}H]lysine 2.5 hours after irradiation.
×: controls;
○: 66 Jm^{-2} UV;
▲: 132 Jm^{-2} UV;
+: 264 Jm^{-2} UV;
▼: 30 krad X-rays;
●: 60 krad X-rays.

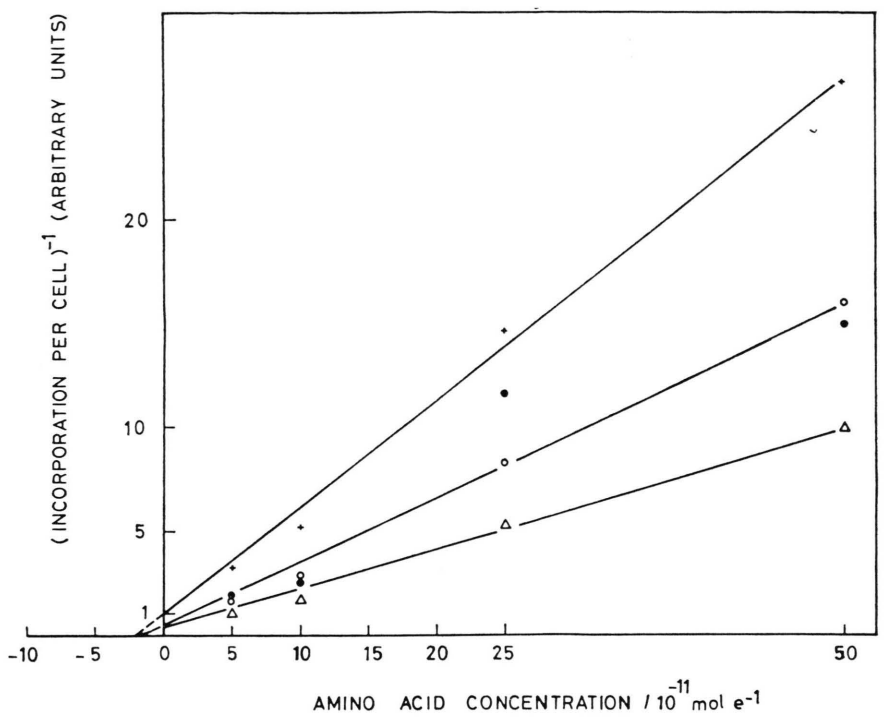

Fig. 3.
Incorporation of [^{3}H]phe 4 hours after UV-irradiation.
+: controls;
○: 66 Jm^{-2};
△: 132 Jm^{-2};
●: 198 Jm^{-2}.

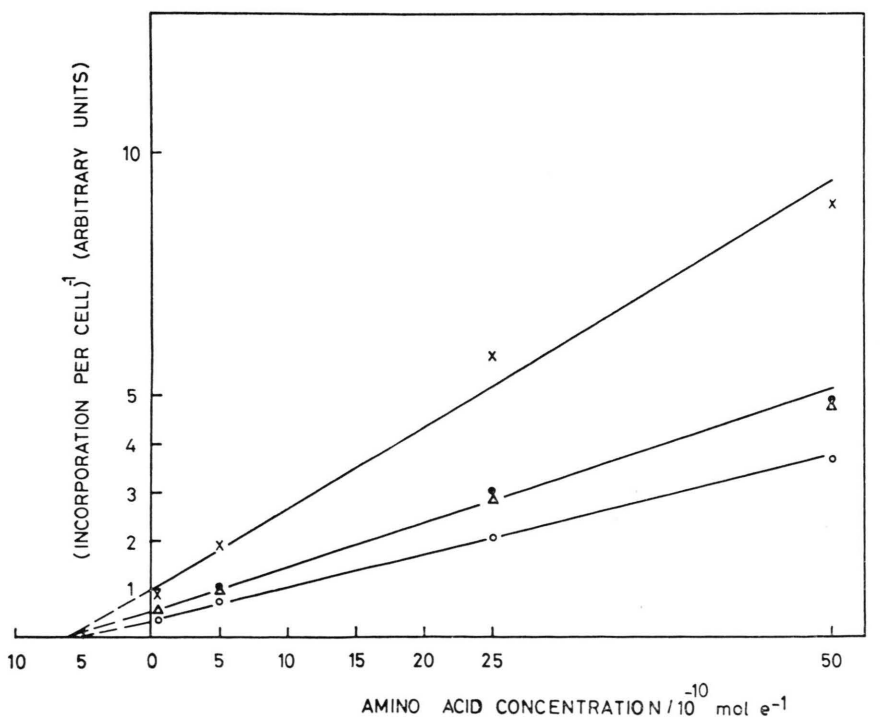

Fig. 4.
As in Fig. 3 but with [^{3}H]lys.
×: controls;
○: 66 Jm^{-2};
●: 132 Jm^{-2};
△: 198 Jm^{-2}.

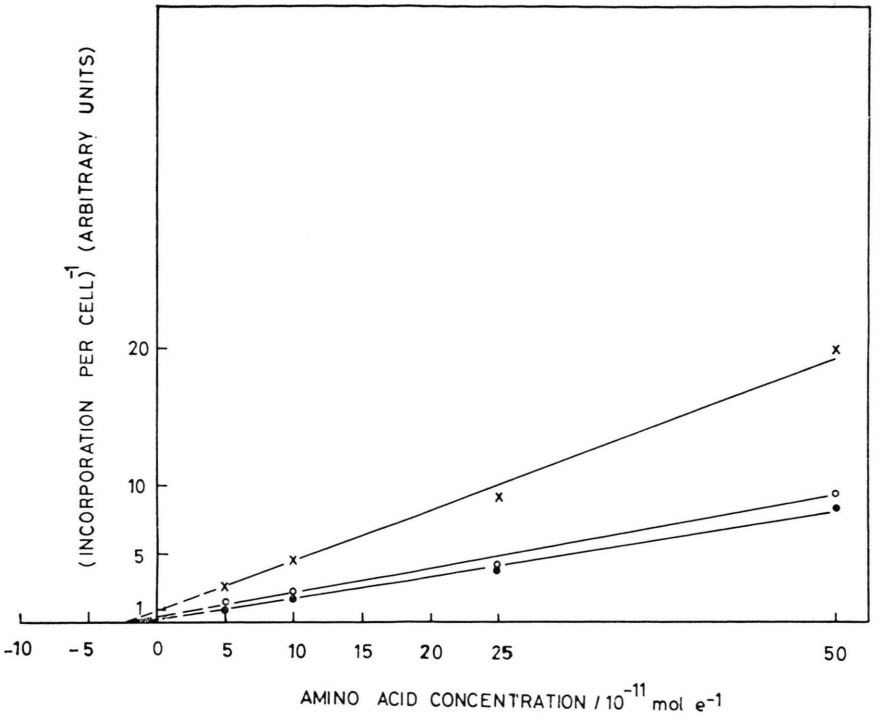

Fig. 5.
Incorporation of [^{3}H]phe 4 hours after X-irradiation.
×: controls;
●: 60 krd;
○: 90 krd.

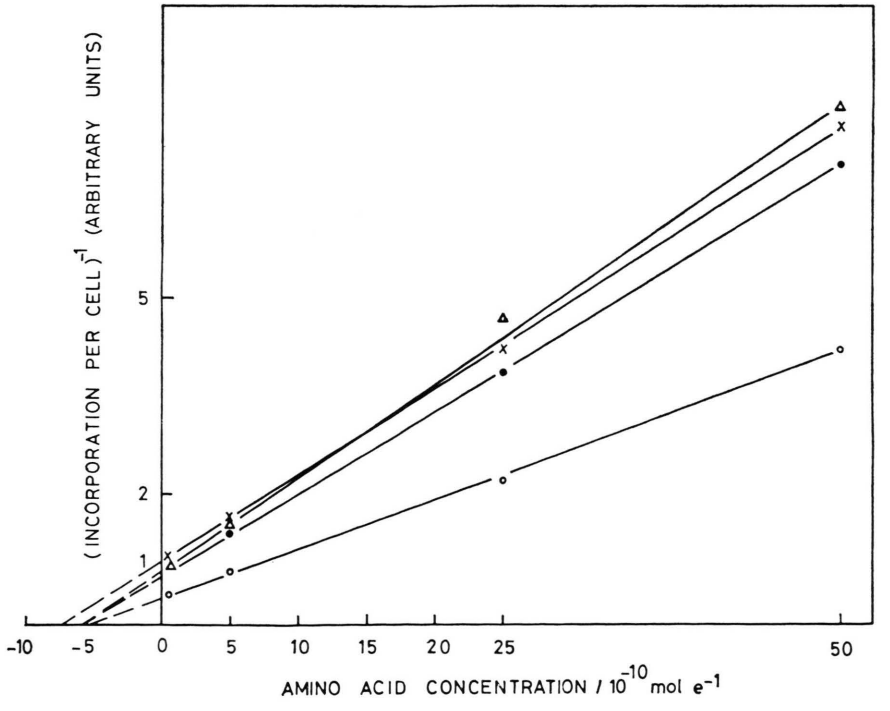

Fig. 6.
As in Figure 5 buth with [^{3}H]lys.
×: controls;
○: 30 krd;
△: 60 krd;
●: 90 krd.

which is still more than the highest exposure applied in the present study. For shorter irradiation times they found an increase in pool sizes in accordance with our results.

Watson [9] determined amino acid pools in *Saccharomyces cerevisiae* under a variety of culture conditions. Since his results are expressed as μmol per dry weight, they are not directly comparable to ours. With asparagine as sole nitrogen source Watson found for phenylalanine a pool size of 0.46 μmol/100 mg dry weight, for lysine the respective figure was 0.73. We measured 2.5 hours after the start of incubation the following a_0/d-values for unirradiated controls:
phenylalanine 5×10^{-11} mol·l^{-1} and lysine 3×10^{-11} mol·l^{-1}.

Although a direct comparison is impossible, it is clear that our figures are much smaller. This suggests that d is greater than unity and that only a minor point of the free amino acids in the cell take part in protein synthesis. The latter statement agrees with findings of Nurse and Wiemken [10] and a suggestion of Watson [9].

The increase in proteins synthesis reported here does not necessarily contradict reports where a reduction was found. Brunschede and Bremer [4] measured a decrease in UV-irradiated *E. coli* but only up to about 30 minutes after exposure. Similar results can be demonstrated in our system [2]. An increase shows up only after longer incubation periods. For X-rays this is also true in mammalian cells [12]. It appears, therefore, that we are no longer dealing with the direct effect of the primary damage but with the radiation-induced disturbance of cellular regulation.

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