Metabolic stability of ribonucleoprotein particles in growing yeast cells

It has been reported that in some non-proliferating animal cells, radioactive amino acids first incorporate most rapidly into microsomal ribonucleoprotein particles, and then move to the soluble proteins of cytoplasm[1,2]. These findings lead one to suppose that the ribonucleoprotein particles are the actual site of cytoplasmic protein synthesis. In microorganisms, particles comparable to those of animal tissues with respect to their chemical and physical properties have been isolated[3,4]. In the experiments described in this paper, the relation between ribonucleoprotein particles and soluble protein synthesis in growing yeast cells has been investigated.

Yeast cells (*Saccharomyces cerevisae*, Strain Fleishman*) grown in glucose-peptone medium were collected and resuspended in synthetic medium[5] at the concentration of 6 mg dry cells per ml. After temperature equilibration at 31° for 5 min, DL-[1-^{14}]alanine (450 μC/mmole) were added at the final concentration of 0.11 mM. The suspension was shaken for 18 min, and chilled by adding crushed ice. After washing with cold water, they were resuspended in synthetic medium containing unlabelled L-alanine (final concn. 2.28 mM), and incubation was continued for the time intervals indicated in Fig. 1. Under these conditions, the amount of protein increases linearly at the rate of 20 %/h. Collected cells were ground with quartz sand for 20 min at 0°, and suspended in the medium of CHAO AND SCHACHMAN[3]. The suspension was centrifuged at 8,000 × g for 20 min to remove large granules, cell wall, and sand. The supernatant was recentrifuged at 105,000 × g for 120 min. The protein samples for radioactivity measurements of both the ribonucleoprotein particles (105,000 × g pellet, 80 S particles of CHAO AND SCHACHMAN[3]) and the final supernatant as well as whole cells were prepared according to ALLFREY *et al.*[6]. It is clear from Fig. 1 that, although the specific activity of the ribonucleoprotein particles was always higher than that of the supernatant (*cf.* Fig. 2), there was apparently no "flow" of radioactivity from the particles to soluble proteins even in the presence

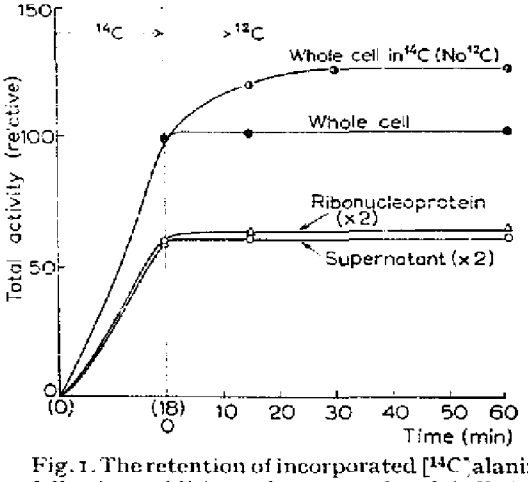

Fig. 1. The retention of incorporated [^{14}C]alanine following addition of excess of unlabelled L-alanine (see text).

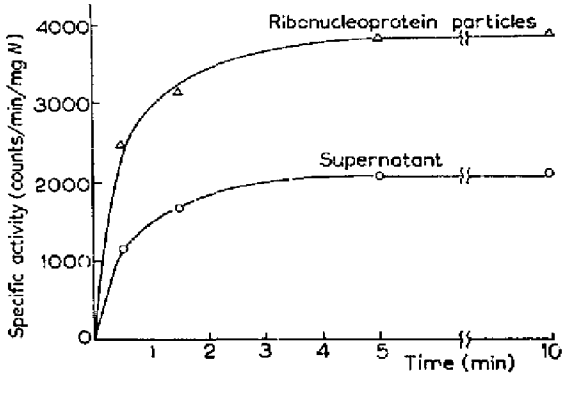

Fig. 2. Incorporation of [^{14}C]leucine into ribonucleoprotein particles and soluble proteins (see text).

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of a large excess of unlabelled alanine. Further purification of the ribonucleoprotein particles by washing, streptomycin precipitation[7], etc. did not change the situation. Experiments of this kind were repeated several times, changing the time of exposure of cells to the [^{14}C]alanine, with similar results. In other experiments, the radioactivity was retained in the ribonucleoprotein particles after suspension of the cells in unlabelled alanine, even after the amount of total protein in culture had increased about five times.

In these proliferating cells, all cellular proteins including ribonucleoprotein are newly synthesized. Therefore, the results indicate that the radioactivity in the ribonucleoprotein particles at the steady state (regarding ^{14}C) is in the constituent proteins of the particle, which thus seem to be metabolically stable once formed. A possibility exists, however, that a small part of the ribonucleoprotein is turning over at an exceedingly high rate to serve as the precursor of soluble proteins. This active part, if it exists, would be detectable by examining the early phase of incorporation with the use of trace amounts of isotope. However, addition of the small quantities of highly radioactive L-leucine, uniformly labelled with ^{14}C (8.78 mC/mmole leucine) to the cell suspension at a final concentration of 2.75 μM resulted in a parallel increase of radioactivity in the ribonucleoprotein and soluble proteins (Fig. 2). Again, no radioactivity "flow" between two fractions were detected. However, the possibility that the active part of the ribonucleoprotein, even if present in intact cells, was removed and transferred to the soluble fraction during the cell fractionation cannot be excluded.

These results suggest, but certainly do not prove, that in growing yeast cells the ribonucleoprotein particles do not serve as the precursor of soluble proteins. Similar views have been presented by ROBERTS, BRITTEN AND BOLTON[8] for the *Escherichia coli* system, using an experimental design different from ours. However, the final conclusion will have to be left until we have a system in which the incorporation of radioactive amino acids into the stable part of the ribonucleoprotein is selectively prevented.

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