EVIDENCE OF HETEROGENEOUS RIBOSOMAL PARTICLES IN SALT-RESISTANT YEAST

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A number of studies has revealed distinguishable ribosomal particles among the cell organella (Lyttleton, 1962; Brawerman, 1963; Boardman et al., 1965, 1966). Besides some evidence has been offered recently which indicates that several types of ribosomal RNA's differing in function may exist in the cell population of a single species (Spiegelman, 1965).

The present author has made an analysis of ribosomal particles derived from an yeast strain cultivated in different salt conditions and observed at least two kinds of ribosomes discriminated from each other by their morphological and functional stabilities under different salt environments. Haploid yeast, Saccharomyces cerevisiae strain R11, which has been acclimatized to a high concentration of $SrCl_2$ (Yamamoto, 1963), has a ribosome very sensitive to salt when it has proliferated in the absence of $SrCl_2$. Alternatively, the same clone produces a salt resistant ribosome under a strontium hypertonicity.

The salt resistant strain R11 which arose from the proliferation under a hypertonic condition (0.75 M $SrCl_2$), incorporated a considerable amount of an amino acid into the cellular proteins in the presence of a high concentration of $SrCl_2$.

When the same strain was precultured in the absence of the salt, the ability of amino acid incorporation in the hypertonic environment disappeared completely. Since the genetic stability of the resistant characteristics through a multiplication without $SrCl_2$ has been confirmed previously (Yamamoto, 1963), it was unlikely that the salt resistant cells were eliminated from the culture by the predomination of salt sensitive revertants. Therefore, the possibility that the salt-resistant yeast could have two sorts of protein synthesizing machineries controlled by the environmental salt concentration was examined.

The R11 cells, grown to the middle of logarithmic growth in the medium with 0.75 M $SrCl_2$, were harvested and resuspended in the medium in the absence of $SrCl_2$. The well-aerated cultivation was carried out for 4 hrs to obtain a duplication of the cell number. An aliquot of the culture was used to determine the changes in amino acid incorporation activity under strontium hypertonicity. The other was diluted to the initial cell concentration by the addition of a same volume of fresh medium and the cultivation was continued. The above described dilution culture without $SrCl_2$ was repeated successively. Fig. 1 shows that the resistant cells originating from a strontium hypertonic condition become more sensitive to salt inhibition in inverse proportion to the number of generation in the salt-free medium. This seems to favour the hypothesis that the salt-resistant amino acid incorporating machinery is induced only in the Sr-hypertonic environment and that its multiplication ceases in the salt-free condition.

If the above consideration is true, some part of the amino acid incorporating machinery should exibit a difference in character with a change of salt environment. Since a difference

has been observed between the ribosomal particles from the Sr-resistant yeast and that of parent strain (Yamamoto,1965), the salt effect on ribosomal particles was investigated. The yeast cells which had been labeled with $^{32}P_i$ (75 mμC/ml) for the first 3 generations in hypertonic $SrCl_2$, were suspended in the Sr-free medium which contained ^{3}H-uracil (1 μC/ml), and thereafter were allowed to multiply for a subsequent 3 generations. 80-S ribosomal particles were obtained from the doubly labeled cells and their morphological stability was

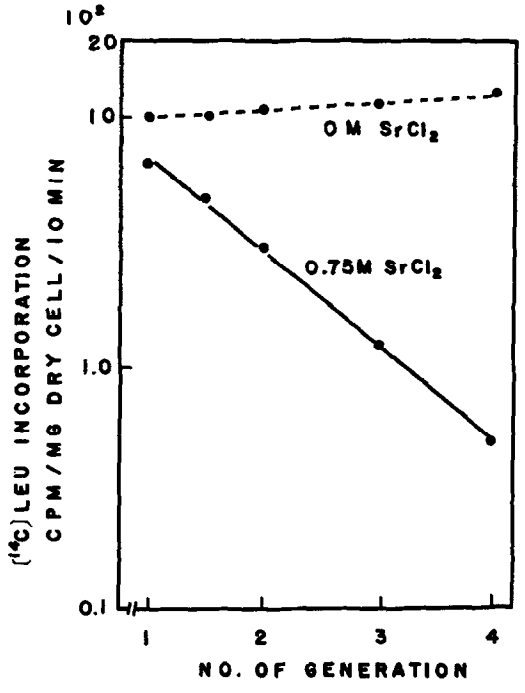

Fig. 1 Effect of high concentration of $SrCl_2$ on amino acid incorporation of yeast cells derived from various preculture conditions. The salt resistant yeast had been grown in hypertonic $SrCl_2$ (0.75 M) and then was made to multiply in the Sr-free medium. Through the proliferation in the latter medium, the constant rate of growth was kept by dilution with the fresh medium at every generation period. The cells from each dilution step were placed in media which contained 0.75 M of $SrCl_2$ or not. 14C-Leucine (0.2 μC/ml; 2 mC/mM) was added after preincubation for 10 min and the incorporation of the amino acid into a cell protein (hot 5% trichloroacetic acid insoluble fraction) was determined. Abscissa indicated the number of generations within the medium free from $SrCl_2$.

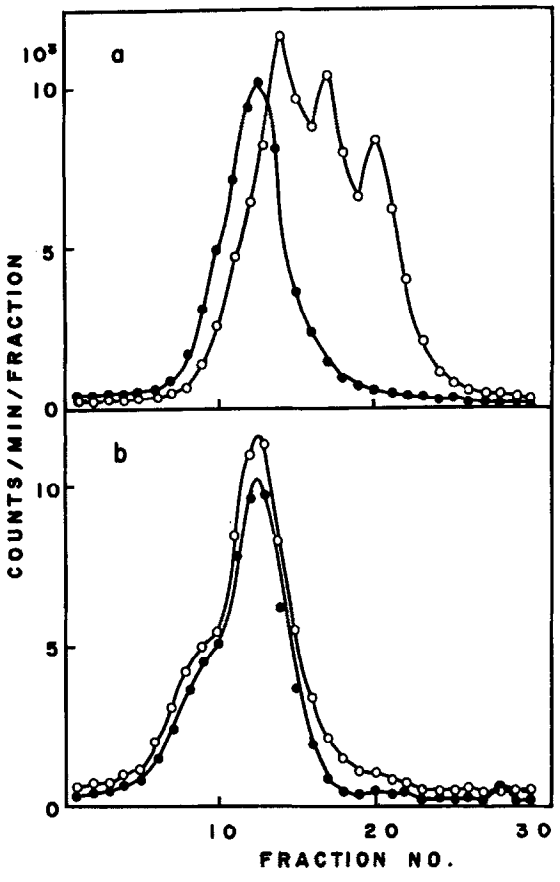

Fig. 2 Sucrose density gradient analysis of doubly labeled 80-S ribosomes.
80-S ribosomal particles were prepared from the cells which had been labeled by either $^{32}P_i$ (○) or ^{3}H-uracil (●) as given in text and they were dialyzed against (a) Tris-HCl buffer, 0.05 M;pH 7.6 with 10 mM of $MgCl_2$ or (b) with 10 mM of $SrCl_2$ overnight at 4°. The centrifugation on a linear sucrose density gradient (5 - 20 %) was made at 37,000 rpm for 90 min with the SW 39 rotor.

investigated by dialyzing them against the different ion concentration (Figs. 2 a and b). The results show that the 80-S particles which have been synthesized in hypertonic $SrCl_2$ are less stable in a medium with 10 mM of $MgCl_2$, while the particles which have been synthesized in the Sr-free condition are not affected. In a more detailed analysis, it was found that the former required at least 7 mM of $SrCl_2$ for stability in vitro and this was not substituted by an excess of Mg^{2+} ion (Yamamoto, 1967).

The specific requirement of Sr^{2+} ion was confirmed for the ribosomal particles which had been synthesized in a strontium

hypertonic condition. A question arose whether these specific ribosomes were responsible for the salt resistant synthesis of cellular proteins or not. The doubly labeled cells were returned to an Sr-hypertonic growing medium again and were aerated for 60 min at 30°. This treatment also ensured the chasing off of previously formed rapidly labeled RNA. A culture was cooled by pouring into 2 volumes of the crushed ice of TMK buffer (M/20 Tris buffer, pH 7.6 containing 5 mM Mg-acetate and 20 mM KCl). After washing twice with a same buffer at 0°, the cells were ground with alumina powder using a precooled pestle and mortar. The cell extract in TMK buffer was centrifuged through a sucrose density gradient (15 - 30 %) at 25,000 rpm for 90 min with the SW 39 rotor.

In front of 80-S monomer peak, only one kind of ribosome, which had been synthesized in the Sr-hypertonic condition, was found (Fig. 3). Since the heavier fractions were eliminated completely by the treatment of RNAase (4 μg/ml for 20 min at 4°) prior to a sucrose gradient analysis, it was identified as a polysomal region. This experiment proved that the ribosome which requires a high concentration of Sr^{2+} ion in vitro was the only active one in polysome formation in the Sr-hypertonic environment. It was also found that the ribosome which was synthesized in the absence of $SrCl_2$ was able to form polysomes in the salt-free medium as well as the Sr-requiring one.

The above-described experiments could clearly discriminate between the ribosome which functions in the hypertonic environment of $SrCl_2$ and that inert in such a condition. It is worthy of note that these differentiated ribosomes are synthesized preferentially under the different cultural conditions in an yeast strain. This means that the yeast strain should have at

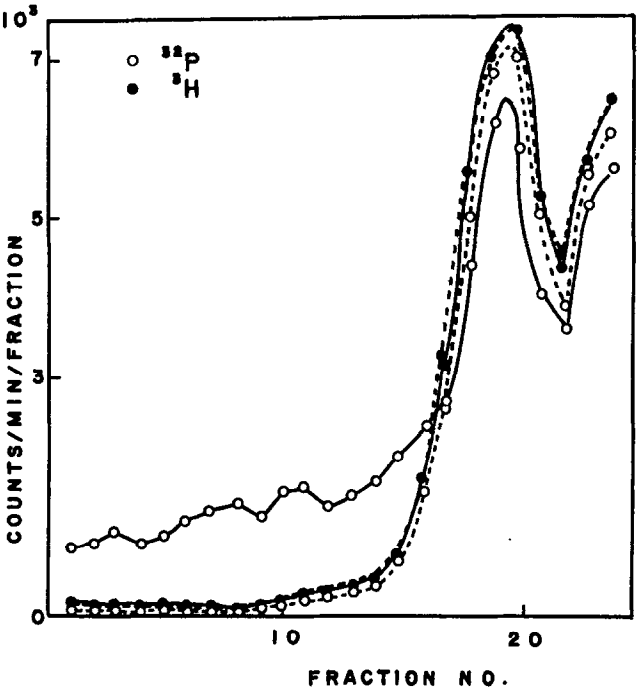

Fig. 3 In vivo polysome formation under high Sr-concentration. The R11 cell was labeled doubly as shown in text. After 60-min incubation in the culture condition with 0.75 M $SrCl_2$, the cells were broken by grinding with alumina powder. The cell extracts were centrifuged on sucrose density gradient (15 - 30 %) at 25,000 rpm for 90 min with or without RNAase pretreatment (4 µg/ml for 20 min at 4°). Solid line indicates non-treatment with the enzyme and dushed one is enzyme treated.

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least two kinds of ribosomal informations and one of them would be activated under the specialized growth condition. The difference in the chemical details of these ribosomes is not yet known and an investigation is in progress.

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