cess L-chain in either case appears as a peak at 3.1*S*.

The area of each peak shows that the amounts of recombined material are essentially equal, and the relative amounts of excess L-chains are as expected (Fig. 1). Therefore, the very low hapten-binding activity for $H_1(X_p)_A$ with $L(X_p)_B$ (Table 1, preparation 5) appears to be due not to a correspondingly low amount of combined material but rather to either a low hapten-binding constant for the material, or to hapten binding by only a small part of the material, or both. The same must also be true for $H_1(X_p)_B$ with $L(X_p)_A$ (Table 1, preparation 7).

Thus the H- and L-chains derived from a pool of specific antibody of several rabbits yield combinations giving good hapten-binding activity, as do those derived from antibody of an individual rabbit. Yet cross-mixing of the H- (or L-) chains from one source with L- (or H-) chains from the other source leads to combination but not to good binding activity. It therefore appears possible that the good hapten binding which results from mixing the H- and L-chains derived from the pool containing serum from the 24 rabbits really represents preferred recombinations between the H- and L-chains originally derived from the antibody of each rabbit contributing to the pool. The H- and L-chains of two or more of the 24 rabbits represented in this pool may possibly cross-combine effectively with one another. However, our results show that neither the H- nor L-chains of at least the one sole rabbit (not in the pool) would combine effectively with the L- or H-chains of an appreciable number of the 24 rabbits making up the pool. Such specificity of combination suggests individual differences among rabbits, and that these differences influence the hapten binding of the combined chains.

We have observed similar specificity of combination between the antibody chains derived from two individual rabbits where *p*-azobenzenearsonate was the hapten. Mixtures of the H- and L-chains derived from the same rabbit gave relatively good recovery of the hapten-binding activity, whereas cross-mixing of the chains of one rabbit with those of another gave little recovery of this activity. However, combination did occur, as judged by radioimmunoelectrophoresis.

Thus mixing H- and L-chains from a pool of antibodies from several rabbits can give a large recovery of sites, and H- and L-chains from different rabbits combine without forming effective sites. These two facts signify that the combinations of chains which give effective sites must be preferred over other combinations.

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9. The H-chains are only slightly soluble in neutral salt solutions. Even in dilute solutions, which are clear initially, precipitate forms. We have measured hapten binding by the part of the H-chain fraction which does not precipitate and even by suspensions of H_2-chains. The binding constant of such preparations is always less than 10 percent of that of the whole antibody from which the H-chain was derived but is, however, higher than normal γ-globulin H-chain controls. The same low binding constants for specific H-chains made soluble with various nonspecific L-chains, as compared with the binding constant of intact antibody, have been obtained many times in this laboratory. Indeed, the H-chains of antibody to *p*-azophenyl-β-lactoside, as reported by Utsumi and Karush [*Biochemistry* **3**, 1329 (1964)], also have values for the binding constant of only about 10 percent of that of the original antibody (mildly reduced).
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Polysomes from Yeast: Distribution of Messenger RNA and Capacity to Support Protein Synthesis in vitro

Abstract. *Individual fractions of polysomes were isolated from yeast. Pulse labeling experiments in vivo show constant specific activity of messenger RNA in each polysome peak; this suggests a uniform density of ribosomes per unit length of messenger RNA. In the cell-free incorporating system, the amount of peptide per ribosome unit increased with the size of polysome.*

In rapidly dividing cells, many of the extracted ribosomes are found in clusters called polysomes. These clusters are of varying size, are apparently bound together by a strand of messenger RNA (mRNA), and serve as templates for protein synthesis. Since cell-free extracts appear devoid of free mRNA and contain an excess of free monosomes (which will rapidly bind added mRNA), the polysome classes in extracts may provide a useful guide to distribution of mRNA molecules with respect to size. Such a study requires high resolution of polysome classes under conditions which minimize mechanical shear and breakdown of RNA by nuclease action. This has been possible in yeast, where improvement in both the extraction of polysomes and analysis by density gradients affords separation and isolation of sufficient quantities of individual classes of polysomes to determine biochemical properties. A rapid turnover of P^{32} and S^{35} occurs in the polysome fraction, and polysomes isolated from sucrose gradients incorporate amino acids, whereas monosomes were inactive unless synthetic mRNA was added (*1*).

We report the size distribution and synthetic activity of the individual polysome classes. In particular an estimate of the ratios of messenger to ribosome to polypeptide was made throughout the polysome fraction.

The hybrid yeast *Saccharomyces dobzhanskii* × *Saccharomyces fragilis* was used. Since stationary cells contain few polysomes, cells were harvested during the log phase of growth by pouring the culture onto ice slivers. All subsequent manipulations were performed at 0° to 4°C to reduce ribonuclease activity. The cells were disrupted by grinding them for 4 minutes with glass beads, and the resulting extract was centifuged (10,000*g* for 10 minutes) to remove debris (*2*). Sucrose gradient sedimentation of this extract yields seven peaks of material heavier than the single 80*S* ribosomes (Fig. 1). Electron micrographs of material from these peaks contain in order, the dimer, trimer, tetramer, and so forth, up to the octomer and

nonamer (*3*). The region of unresolved polysomes sedimenting ahead of the fastest peak contains polysomes consisting of 10 to 20 ribosomes. In this

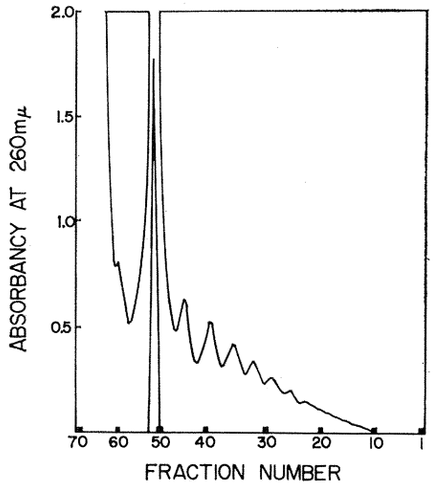

Fig. 1. Sedimentation pattern of ribosomes from extracts of yeast. Portions (0.5 ml) of cell-free extracts of exponentially growing yeast were sedimented through a 7- to 37-percent exponential sucrose density gradient (*8*) for 3½ hours at 24,000 rev/min in a Spinco model L-SW 25 rotor. After centrifugation the contents of the tube were drained through a flow cell and monitored continuously at 260 mμ. The flow cell was first filled with 40 percent sucrose, and the flow path from the centrifuge tube through the flow cell was continuously descending; this prevented convective mixing caused by density inversion in ascending paths.

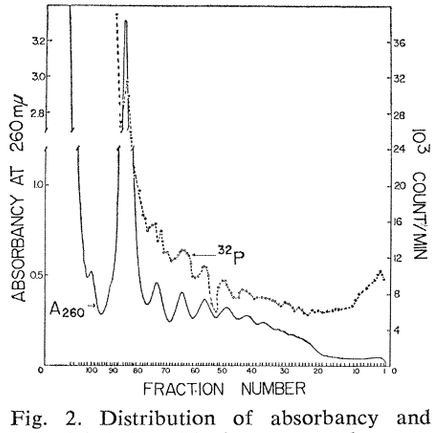

Fig. 2. Distribution of absorbancy and pulse-labeled RNA in yeast polysomes. The yeast culture, grown in a low-phosphate (50 μg/ml) synthetic medium supplemented with vitamins (*9*), was pulse-labeled for 90 seconds and poured onto ice; extract was prepared as already described. After centrifugation and during continuous monitoring at 260 mμ, fractions (15 drops each) were collected directly into counting vials. Scintillation fluid was added and the fractions were measured for radioactivity in a Packard Tri-Carb spectrometer.

experiment, polysomes make up about 50 percent of the total ribosomal population. Cell extracts containing 70 percent polysomes have been obtained.

To characterize and determine the distribution of fast-labeling RNA, yeast cultures were briefly exposed to P^{32}-labeled phosphate, the exposed cells were disrupted, and the polysomes were fractionated on a sucrose density gradient. The polysomes were extracted with phenol or lithium chloride, and the RNA so obtained was then chromatographed on methylated albumin Kieselguhr columns. Much of the radioactivity was identified as mRNA, in that it is eluted after the 16*S* and 23*S* ribosomal RNA in the region normally associated with mRNA, and has a base ratio approaching that of DNA (*4*). The distribution of mRNA among the various sized polysomes is seen from sucrose density gradient patterns of extracts of cells pulse labeled with phosphate-P^{32} (Fig. 2). Quantitative comparison of this distribution is shown in Fig. 3. That the specific activity is essentially invariant indicates a constant density of ribosome per unit length of messenger. Similar distributions have been observed for rapidly labeling RNA and protein in other systems (*5*). These data imply uniform distribution of ribosomes on mRNA, whereas the Ames and Hartman model of message modulation (*6*) provides a mechanism by which the ribosomal density could decrease with increasing message length.

The radioactivity in the monosome peak (Fig. 2) is in part due to the diffusion of phosphate contaminants of low molecular weight into the 80*S* region. Extended sedimentation sometimes permitted isolation of the monosome peak. The specific activity of the monosomes in pulse-labeled cells, after precipitation with 10 percent cold trichloroacetic acid, ranged from one-fourth to one-third of that in the polysome region. This radioactivity could reflect the fraction of monosomes attached to fragments of mRNA or could result from ribosomal RNA synthesized during the labeling period. Neither relative rates of synthesis of ribosomal RNA and mRNA nor the half life of mRNA in yeast have been determined.

To test whether the monosome peak contains mainly free ribosomes, whereas the polysome fractions contain ribosomes connected by mRNA, we examined their capacity to support

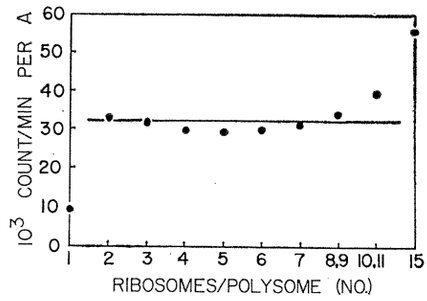

Fig. 3. Relation of specific activity from rapidly labeling RNA to polysome size class. Radioactivity to ultraviolet absorbancy ratios from Fig. 2 are plotted against number of ribosomes per polysome. Electron microscopy and sedimentation velocity identified tube 85 as single ribosomes, tube 75 as dimers, 66 as trimers, and so forth. Tubes 0 to 10 are not evaluated because these rapidly sedimenting aggregates have not been characterized, and turbidity made the absorbancy data uncertain.

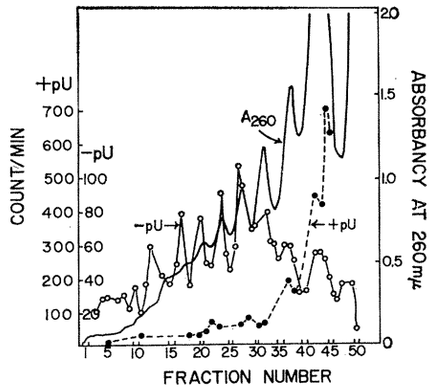

Fig. 4. Phenylalanine-C^{14} incorporation by isolated ribosome fractions. Polysomes were isolated as described (Fig. 2). The yeast cell-free incorporating system of Bretthauer *et al.* (*2*) was used; the incubation period was 60 minutes, in all cases long enough to give maximum incorporation. Addition of C^{12} amino acids to the incorporating system did not increase activity.

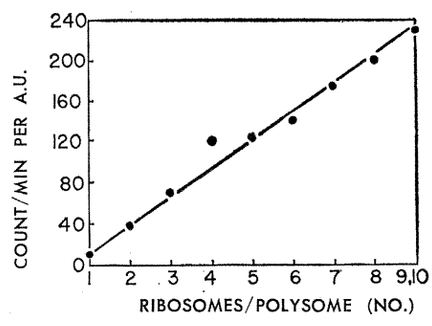

Fig. 5. Relation of specific activity of C^{14}-phenylalanine incorporation to polysome size class.

protein synthesis in the absence of added mRNA (Fig. 4). The curve for absorbancy shows the separation of polysomes into at least seven classes with respect to size. The amount of amino acid that had been incorporated was determined for each class. The single ribosomes exhibited one-tenth to one-twentieth the specific activity of the larger polysomes, and this suggests that some of the ribosomes still contain mRNA or fragments of mRNA. These fragments support amino acid incorporation in this system (*1*). As expected, synthetic mRNA (polyuridylic acid) primarily stimulates only the single ribosomes in which C^{14}-phenylalanine incorporation rose about 15-fold.

The amount of labeled amino acid incorporated can be used as a measure of the accumulation of peptide product on the ribosomes. Plotting the specific activity of each peak fraction against polysome size (Fig. 5) shows regularly increasing amounts of peptide per ribosome unit as the polysome size increases. This is the predicted result if the larger polysomes contain longer strands of mRNA and produce higher molecular weight protein. From the theory of modulation of polycistronic messages by release of product protein by ribosomes at the end of a cistron (*7*) one can predict that the specific activity would increase at a diminishing rate as the polysome size increased. In the *Escherichia coli* system which was first depleted of basal mRNA and then supplemented with polycistronic messenger RNA from turnip yellow mosaic virus, the amino acid incorporation per ribosome particle rose initially, remained generally constant up to the octomer, and then decreased (*8*). In our experiments the largest polysome clusters were of such size that many clusters would contain more than one cistron per mRNA. In these large polysomes the rate of increase of specific activity with polysome size was undiminished. Our results could lose some of their significance if the mechanism of regulation responsible for polycistronic controls is not preserved by the system in vitro.

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Antibodies against the Component Polypeptide Chains of Bovine Insulin

Abstract. *Antibodies were produced in guinea pigs against A and B chains of bovine insulin; the chains were prepared by cleavage of all the disulfide bonds with sulfite. Antibodies against A chain reacted with A chain, but not with B chain or native insulin. Antibodies against B chain reacted strongly with both B chain and insulin, but not with A chain. Antibodies against insulin reacted strongly with insulin, weakly with B chain, and not at all with A chain. The results indicate that the insulin-reactive antibodies in antiserums to B chain and to insulin differ and are directed to differing individual antigenic regions of the insulin molecule.*

The primary structure of bovine insulin is well known; insulin is composed of two polypeptide chains connected by two interchain disulfide bonds (*1*). The A chain consists of 21 amino acid residues with *N*-terminal glycine and *C*-terminal asparagine and has one intrachain disulfide bond between the two cysteine residues at 6 and 11. The B chain consists of 30 amino acid residues with *N*-terminal phenylalanine and *C*-terminal alanine. Insulins of other species of mammals as well as of fishes have chains closely related in structure (*2*). Antibodies against these component chains of insulin, which are of such a small molecular size and of known amino acid sequence, could serve as models for studying the interaction of more complex protein antigens and antiprotein antibodies. In addition, these antibodies should be useful in biological studies of insulin and perhaps of the insulin antagonist in serum (*3*).

We now report on the antibodies produced in guinea pigs against the A and B chains of bovine insulin. The chains were obtained by cleavage of all the disulfide bonds with sulfite. Antigens labeled with I^{125} were used to detect antibodies. Antigens bound to antibodies were separated from free antigens by precipitation with rabbit antiserum against components of guinea pig serum.

Crystalline bovine zinc insulin (*4*) was freed of most zinc by isoelectric precipitation in the presence of ethylenediamine tetraacetate. The product was submitted to chromatography on diethylaminoethyl cellulose in *tris*-phosphate buffer containing 6*M* urea (*5*) at 5°C to remove desamidoinsulin and two minor contaminants (*6*). The purified insulin showed a sharp single band on electrophoresis on paper in veronal buffer containing 6*M* urea (*p*H 8.6, $\Gamma/2 = 0.075$) and on disc electrophoresis at *p*H 8.9 (*7*).

The *S*-sulfonated A and B chains (SS-A and SS-B chains) were prepared from the chromatographically purified bovine insulin with sulfite and tetrathionate at *p*H 7.4 in 6*M* urea (*5*) in the manner of Bailey and Cole (*8*), with minor modifications. On paper electrophoresis in urea-veronal buffer, the SS-A chain gave a single band of fast mobility; the SS-B chain showed essentially a single band at a position slightly slower than that of insulin. Occasionally, another component was observed migrating more slowly in small quantity. The results of amino acid analysis were in good agreement with the values expected for the sulfonated chains. Threonine, proline,