Breakdown and Assembly of Polyribosomes in Synchronized Chinese Hamster Cells

Abstract. *Polyribosomes were absent in metaphase Chinese hamster cells and were reassembled after mitosis. This assembly correlated temporally with formation of nuclear membrane and decondensation of chromatin, accounted for a threefold increased rate of protein synthesis, and occurred independently of* de novo *RNA synthesis, that is, it utilized preexisting messenger RNA.*

Knowledge of mechanisms which control macromolecular synthesis during the life cycle of mammalian cells is of prime importance toward understanding cellular proliferation. Autoradiographic analyses clearly demonstrate that during mitosis there is no DNA synthesis, a cessation of RNA synthesis, and a rapid decrease in the rate of protein synthesis (*1*). While the lack of RNA and DNA synthesis may be attributed to the condensed state of the chromatin at mitosis, an explanation for the reduced rate of protein synthesis is not so apparent. Recent evidence has established that the physical and catalytic site of nascent protein synthesis in the cell is the "polyribosome," an aggregate of two or more ribosomes bound to a strand of "messenger RNA" (*2*). Scharff and Robbins showed that polyribosomes disaggregate during metaphase in HeLa cells (*3*), and Salb and Marcus demonstrated that metaphase ribosomes were the rate-limiting component in a polyuridylic acid–directed, cell-free, amino acid–incorporating system (*4*). The present experiments were performed by using synchronized Chinese hamster cells to determine if the assembly of polyribosomes after mitosis involved preexisting messenger RNA and to precisely coordinate the appearance of polyribosomes with other events in the cell cycle.

Don C Chinese hamster cells grown in monolayers have about a 12-hour generation time (*5*). Synchronous cells (1 to 2 × 10^8) were obtained by selective detachment of cells arrested in metaphase by colcemid (0.06 μg/ml) from monolayers grown in large rotating glass cylinders (*5*). In order to determine the incorporation of labeled amino acids into nascent polypeptides, suspension cultures of synchronized cells were pulse labeled for 1 minute with a C^{14}-amino acid mixture (2 μc/ml, about 1 mc/mg, New England Nuclear Corp.) (*6*). Cells were collected by centrifugation and cell lysates prepared as described previously (*3*). The polyribosome size distribution in cell lysates was determined by zone sedimentation analysis in "constant velocity" sucrose gradients (*7*). This method allowed direct and reasonably precise measurement of sedimentation coefficients of various ribosomal species. After ultracentrifugation the contents of gradients were analyzed continuously for ultraviolet-absorbing material at 260 mμ. Gradient fractions were collected, made 1*N* in NaOH for 3 minutes, and 10 percent trichloroacetic acid was added in excess. Trichloroacetic acid precipitates were collected and washed on 0.45-μ cellulose nitrate filters and assayed for C^{14} radioactivity in a liquid scintillation spectrometer.

The polyribosome size distributions (Fig. 1) found in cell lysates demonstrate that metaphase cells (97 percent mitotic index, Fig. 1b) showed less ultraviolet-absorbing material in the polyribosome region of the gradient (119 to 300*S*), smaller polyribosomes, and more ultraviolet-absorbing material in the single 80*S* ribosome peak than did interphase cells (4 percent mitotic index, Fig. 1a). Moreover, in metaphase cells there was approximately a 75 percent reduction in the amount of C^{14} radioactivity found in the polyribosome region of the gradient. Although metaphase cells contained predominantly 80*S* ribosomes, these were not nearly as active in protein synthesis as the polyribosomes remaining (Fig. 1b). These data agree with an earlier report based on the use of HeLa cells (*3*). Incubation of portions of the initial metaphase population in medium without colcemid resulted in the synchronous progression of cells out of mitosis and into the postmitotic (G_1) phase of the cell cycle (*5*). After a half hour's incubation, the population consisted primarily of cells in anaphase and telophase; after 1 and

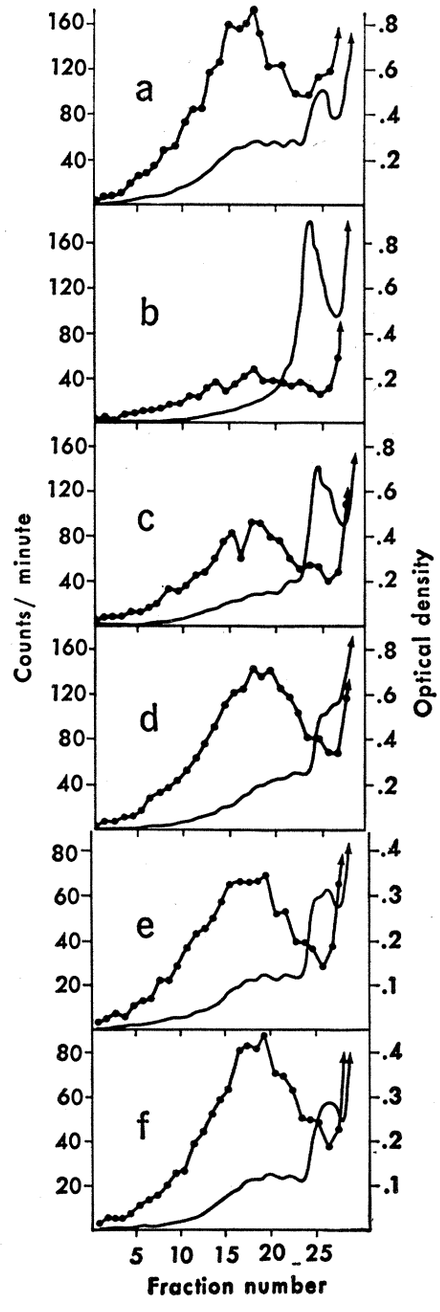

Fig. 1. Polyribosome reformation of postmitotic cells. An interphase cell suspension (obtained by trypsinization of that portion of the asynchronous population remaining after selection of the metaphase cells) was incubated for 0.5 hour and pulse labeled for 1 minute with a C^{14}-amino acid mixture (a). A metaphase cell suspension was distributed into three equal samples. One sample (b) was suspended in medium containing 0.06 μg of colcemid per milliliter, incubated for 0.5 hour, and pulse labeled as described in the text. The other two samples were suspended in medium without colcemid, incubated for 0.5 hour (c) and 1 hour (d), respectively, at 37°C, and also pulse labeled. Replicate samples of metaphase cells were incubated, at 37°C, for 2 hours in medium without inhibitors (e) and 2 hours in medium containing 5 μg of actinomycin D per milliliter (f) and pulse labeled as above. Cell lysates of each sample were centrifuged for 3.5 hours in exponential constant velocity sucrose gradients. After centrifugation the gradients were analyzed for ultraviolet-absorbing material at 260 mμ (plain lines) and C^{14} radioactivity (lines with circles).

2 hours, the percentage of cells in G_1—those having decondensed chromatin and a formed nuclear membrane—was routinely about 88 and 94 percent, respectively. Subsequent analysis on sucrose gradients (Fig. 1, b, c, and d) showed there was a gradual increase in the size and amount of polyribosomes and a simultaneous decrease in the amount of 80*S* ribosomes with increasing time of incubation of the initial metaphase population. The amount of C^{14} radioactivity increased in proportion to the optical density in the polyribosome region. The size distribution of polyribosomes at 2 hours after mitosis (Fig. 1e) approached that found in asynchronous populations (Fig. 1a). Polyribosome assembly after mitosis correlated temporally with decondensation of chromatin and formation of nuclear membrane.

The observed assembly of polyribosomes was correlated with the rate of protein synthesis in cells at different times after mitosis (Fig. 2). The rate of protein synthesis more than tripled during the first 2 hours of incubation of initially metaphase populations. While this increase in the rate of protein synthesis was reflected in a similar increase in the specific activity of total ribosomes, the specific activity of polyribosomes was invariant (Fig. 2). These results clearly demonstrate that the increase in the rate of protein synthesis after mitosis can be accounted for by an increase in the number of ribosomes found in polyribosomes.

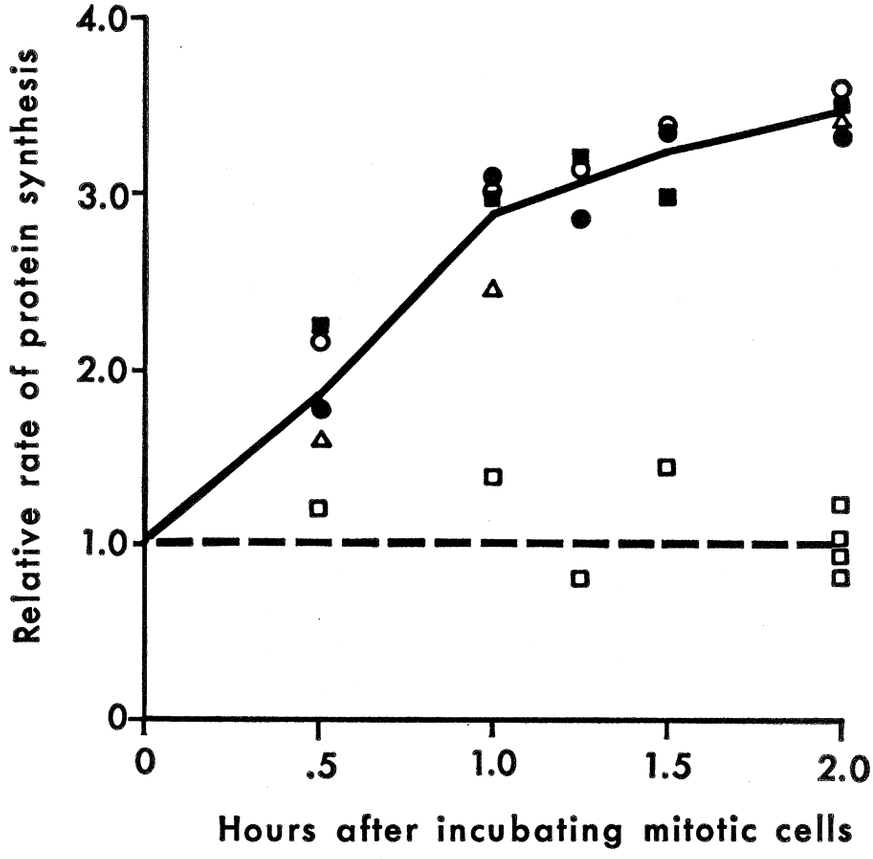

Fig. 2. Relative rate of protein synthesis at different times after incubating metaphase populations. The relative rate at each time point is expressed as a ratio of the rate of protein synthesis in postmitotic cells to that in the initial metaphase population in the same experiment. The rate of protein synthesis in whole cells was expressed as the amount of C^{14}-amino acids incorporated into trichloroacetic acid–precipitable samples of the cells during a 15-minute pulse label (△). The following determinations were made on the sucrose gradient centrifugates of cytoplasmic extracts from mitotic and postmitotic cells which had received a 1-minute pulse label with C^{14}-amino acids. The total C^{14} incorporation in all fractions from a sucrose gradient was measured (○). The protein radioactivity of the sucrose gradient fractions which sedimented slower than the 80*S* monosome peak (soluble protein) was determined (■). The specific activity of total ribosomes was determined as the ratio of total counts per minute of C^{14} radioactivity to total optical density of material sedimenting with and faster than the single 80*S* monosome peak in sucrose gradients (●). Solid lines connect points which are averages of the above values. The specific activity of polyribosomes (□) was determined as above for material sedimenting with and faster than the 119*S* dimer peak in sucrose gradients (dashed line).

Since the possibility existed that the reduced rate of protein synthesis and breakdown of polyribosomes observed at mitosis could be due to a loss of messenger RNA it was important to determine whether polyribosome assembly after mitosis was dependent on *de novo* RNA synthesis. Actinomycin D, a known inhibitor of DNA-dependent RNA synthesis (*8*), was added to the medium during incubation of initially metaphase populations. Figure 1, e and f, shows that the polyribosome size distribution and associated C^{14} radioactivity were similar in postmitotic cells incubated for 2 hours after mitosis with and without 5 μg of actinomycin D per milliliter. In this experiment the control gradient from the initial metaphase sample was similar to that shown in Fig. 1b. Data not shown indicated that under these same conditions 5 μg of actinomycin D per milliliter inhibited the incorporation of H^3-uridine into acid-precipitable fractions of (i) whole cells by at least 93 percent and (ii) ribosomes and polyribosomes by 99 percent. These results demonstrate that polyribosome assembly after mitosis occurs independently of *de novo* RNA synthesis, suggesting that messenger RNA is passed from mother cells to daughter cells and is used to direct protein synthesis during at least the initial stages of the ensuing cell cycle.

Consideration of the currently accepted model for polyribosome function during protein synthesis suggests that a gradual increase in the rate of attachment of ribosomes to existing messenger RNA would account for both the increase in size and amount of polyribosomes and the increased rate of protein synthesis as cells move out of mitosis and into the ensuing G_1 phase. Several investigators have speculated that the rate of attachment of ribosomes to messeger may be the rate-limiting step in protein synthesis in the intact cell (*9*). Lin and Key recently presented evidence which suggested that polyribosome breakdown, induced in soybean root by anaerobic conditions or treatment with dinitrophenol, was associated with a reduction in the rate of attachment of ribosomes to messenger RNA (*10*). While a decrease in the rate of movement of ribosomes down messenger would also account for the observed increase in the size and amount of polyribosomes,

this would not account for the increased rate of protein synthesis observed after mitosis.

The system presented here offers potential for study of macromolecular events in mammalian cells having a high degree of synchrony during a particular stage of the cell cycle.

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6. In experiments on asynchronous populations it was found that pulse-labeling with C^{14}-amino acids for periods longer than 1 minute did not appreciably increase the C^{14} radioactivity in the polyribosome region of gradients but almost linearly increased the C^{14} radioactivity found near the top of the gradients. These results suggest that the 1-minute pulse label was sufficient to achieve steady state labeling of the growing polypeptide chains associated with polyribosomes.
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Remission of Aster Yellows Disease by Antibiotics

Abstract. *Suppression of symptoms of aster yellows by antibiotics supports the tentative hypothesis that the etiologic agent is a mycoplasma- or bedsonia-like organism rather than a virus. Development of symptoms was supressed by chlortetracycline, tetracycline, or chloramphenicol, but not by penicillin. When plants were treated with chlortetracycline at 1000 parts per million before symptoms appeared, symptoms developed only after cessation of the treatment. Assay of the agent of aster yellows, extracted from plants, indicated inhibition of growth of the pathogen by treatment with chlortetracycline. Plants severely affected before treatment began developed new symptomless axillary growth, including flowers; previously yellowed leaves often became green. Acquisition of the agent of aster yellows by leafhoppers was drastically reduced when infected plants were treated with chlortetracycline continuously for 1 week before exposure to the vectors. Our data, and preliminary evidence from purification studies, are consistent with a possible mycoplasma- or bedsonia-like etiology of the aster yellows disease.*

The causal agents of aster yellows, and similar plant diseases borne by leafhoppers, have long been considered viruses, a supposition based mainly on their filterability and their transmittability by grafting; yet they have long resisted isolation and visualization. Failures in purification have often been ascribed to instability in vitro, low concentration in infected tissues and cumbersome bioassay, but now it appears that the pathogen may not be a virus.

Japanese workers (*1*, *2*) proposed that yellows diseases of plants may be caused by mycoplasma- or psittacosis-like (bedsonia) organisms; their evidence was based on electron microscopy of diseased tissues and a therapeutic effect of tetracycline antibiotics. Early results (*3*) on sedimentation and filterability of the aster yellows agent, later work (*4*), and our own data from purification studies could be explained by a mycoplasma-like etiology of aster yellows.

We have examined this hypothesis with aster yellows. Here we summarize data from chemotherapy experiments (*5*) based on the tenet that aster yellows, if caused by a mycoplasma or bedsonia organism, should be amenable to treatment with certain "broad-spectrum" antibiotics.

A celery-infecting strain of aster yellows and the vector *Macrosteles fascifrons* Stål were used throughout. Hydroponics experiments were performed in a controlled-environment chamber at 27°C with a 16-hour day and an 8-hour night. In other experiments, plants were maintained in a greenhouse at 23° to 27°C.

In our first experiments, small plants of annual chrysanthemum (*Chrysanthemum carinatum* Schousb.) were treated with antibiotic before symptoms appeared. Leafhoppers, after 3 days of feeding for inoculation, were removed from the plants and the roots were washed and treated immediately (or 3 or 6 days later) by immersion for 10 minutes in either chlortetracycline (*6*) or buffer. When plants were repotted in soil, the respective antibiotic or control solution was applied to the foliage in a fine spray (to run-off) at 3-day intervals.

Four separate experiments gave comparable results. Symptoms of aster yellows in plants treated with chlortetracycline at 1000 parts per million (ppm) appeared only when spraying was discontinued (Table 1). Chlortetracycline at 100 ppm also suppressed development of symptoms but some plants, first treated 9 days after exposure to infectious vectors, developed symptoms as soon as did control plants. Penicillin (*7*), on the other hand, had no effect on development of symptoms.

Chloramphenicol (*8*) and tetracycline (*9*) were tested in the same way in five separate trials; all results were comparable (Table 2). Development of symptoms was suppressed by either tetracycline or chloramphenicol, the latter being much less efficacious.

Aster plants having advanced symptoms of aster yellows were then placed in hydroponic culture solution, with or without chlortetracycline (10 ppm). All but one chlortetracycline-treated plant produced new symptomless axillary growth; eventually one plant produced six flower buds that opened to display symptomless flowers (Fig. 1). Control plants developed no new growth during the experiment, and there was no greening of already yellowed leaves.

Suppression of development of symptoms, and remission of existing symptoms of disease, by application of chlortetracycline to foliage, without prior dipping of the roots, was demonstrated in several preliminary experiments with aster, annual chrysanthemum, and celery (*Apium graveolens* L.); treatments were begun within 1 week after appearance of symptoms, so that the plants were much less severely affected by the aster yellows than those used in the