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Quantitative Analysis of the Heat Shock Response of *Saccharomyces cerevisiae*

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Transient protein synthesis in *Saccharomyces cerevisiae*, after shift from 21–23°C to 37°C, was quantitatively analyzed. Pulse-labeled proteins were separated by two-dimensional gel electrophoresis, and autoradiograms of the gels were analyzed by a recently described method involving a computer-coupled film scanning system. In this way, the rate of incorporation of L-[^{35}S]methionine into approximately 500 proteins was followed. The synthesis of more than 80 of these proteins was transiently induced at 37°C, with about 20 being classified as major heat shock proteins (defined as those whose rate of labeling was increased at least eightfold at some time during the response). The synthesis of more than 300 of the proteins was transiently repressed at 37°C, and several general temporal patterns of repression could be distinguished. The influence of temperature-sensitive mutations affecting RNA synthesis and transport on the heat shock response was also examined. A protein whose induction in response to heat shock has a post-transcriptional component could be identified. As previously pointed out, the heat shock repression of certain proteins is so rapid that it also must involve post-transcriptional effects.

The heat shock response appears to be universal. First discovered in insects (35), it is manifested, at the cellular level, in animals (16), plants (18), fungi (28, 30), and other higher protists (e.g., 9, 24, 43, 47) and bacteria (21, 46; for reviews, see references 3 and 34). The cellular response to a temperature up-shift involves the transient induction of synthesis of a characteristic set of proteins and the transient repression of synthesis of many (16, 21) or almost all (22, 40) others. The response involves transcriptional (4, 13, 28, 32, 37) as well as post-transcriptional (31) mechanisms (the latter have been analyzed in some detail in connection with the response in *Drosophila* spp. [20, 38]) and also (post-translational) modification of certain proteins (11, 24a, 44). At least a part of one of the heat shock proteins is very highly conserved in evolution. Antibody to a chicken heat shock-induced 76-kilodalton protein (16) cross-reacts with heat shock-induced proteins from *Dictyostelium discoideum* (17; W. F. Loomis, personal communication) and yeasts (17; W. F. Loomis and M. J. Miller, unpublished data) of comparable molecular weights. At the functional level, the heat shock response appears to protect cells from killing at high temperatures (24, 26).

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We studied protein synthesis in *Saccharomyces cerevisiae* in a transient period after shift from 21–23°C to 37°C. The pattern of changed protein synthesis is relatively complex; although the synthesis of many proteins is induced, many others are repressed and still others are not appreciably changed. In this paper, we present a relatively detailed analysis of the largely transient changes that occur in the synthesis of several hundred proteins as a result of temperature up-shift. For this analysis we used two-dimensional gel electrophoretic separation of briefly labeled proteins and analyzed the autoradiograms of such gels by a recently described procedure (42).

MATERIALS AND METHODS

Cells, media, temperature shift, labeling, and sample preparation. *S. cerevisiae* M25, a diploid, originally provided by J. Bossinger and T. Cooper (5, 45), whose genotype is

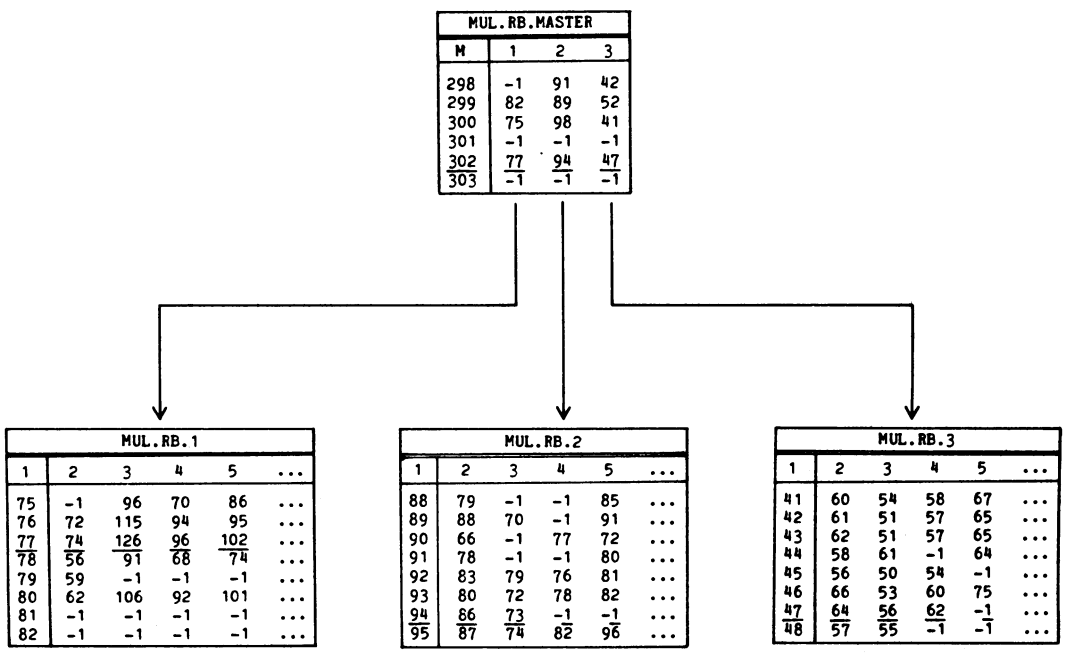
$$\frac{\mathbf{a}}{\alpha}\ \frac{his6}{+}\ \frac{ura1}{+}\ \frac{lys2}{+}\ \frac{+}{ade6}\ \frac{+}{leu1}$$

was used in all of the experiments. Experiments with diploid strains M304 and M421 are also referred to in the text. Strain M421 was provided by T. Cooper and is the subject of further analysis (M. J. Miller, N. H. Xuong, and T. Cooper, manuscript in preparation). Strain M304 is homozygous for the *rna1* mutation (15), which affects some RNA processing (14). Strain M421

is homozygous for one of the RNA synthesis mutations (*ts4472*) described by Thonart et al. (39). Both mutations block the appearance of new mRNA in the cytoplasm at 37°C (36). Cells were grown in MV-A minimal medium (12) supplemented with 20 μg of L-tyrosine per ml, which stimulates the incorporation rate of L-[^{35}S]methionine, as previously recorded (30). Cells were grown at 21 to 23°C, transferred to 37°C, and labeled with carrier-free L-[^{35}S]methionine as described previously (30). Labeling (5-min intervals) was initiated by the addition of ^{35}S-labeled amino acid to the medium and was terminated by the addition of unlabeled L-methionine to a final concentration of 1 mM for 1 min. Cells were then cooled rapidly, centrifuged, washed once with 20 mM Tris-chloride (pH 8.8)–2 mM $CaCl_2$, recentrifuged, frozen, and kept at −70°C until ready for further processing. Labeled cells were disrupted, treated with nucleases, lyophilized, and suspended in buffer A, described by O'Farrell (33), at a protein concentration of ca. 3 mg/ml. Incorporation of ^{35}S into protein and protein concentration were determined immediately after cell disruption. Radioactivity was determined by precipitating with 10% Cl_3CCOOH in the presence of 250 μg of bovine serum albumin carrier, boiling for 10 min, cooling, filtering the precipitated protein on glass fiber filters (Whatman, GF/C), and counting in an NCS solubilizer (Nuclear-Chicago)-containing, toluene-based scintillation fluid. The counting efficiency of ^{35}S in this medium, checked periodically, was 85 ± 5%. Protein concentration was measured by the method of Lowry et al. (25).

The following parameters for ^{35}S labeling, at 22°C, were determined in control experiments. Incorporation of L-[^{35}S]methionine showed a slight lag (extrapolating to ca. 1.5 min) and then was linear for ca. 40 min. Incorporation in 5- or 10-min pulses was proportional to an input radioactivity of up to 30 to 100 μCi/ml; the amount of label used in these experiments was within that linear range, varying between 4 and 15 μCi/ml. L-[^{35}S]Methionine was purchased (New England Nuclear Corp.) or made from hydrolyzed $^{35}SO_4$-labeled *Escherichia coli* by the method of Bretscher and Smith (8).

Two-dimensional gel electrophoresis was carried out essentially as described by O'Farrell (33), but with minor variations. Samples were run in 9.65 M urea on two isofocusing gels covering, respectively, nominal pH ranges 5 to 7 and 6 to 8 (10). Isofocusing tube gels (2.2-mm diameter) were made with the aid of the gel-casting apparatus described by Anderson and Anderson (2). The proteins were separated in the second dimension on 10% polyacrylamide slab gels, using stacking gel as described by O'Farrell (33). At the upper surface, the stacking gel was polymerized against a glass rod (3-mm diameter) to provide a smooth interface with the cylindrical isofocusing gel. The latter was placed directly onto the stacking gel and held in place with a small amount of agarose. Autoradiograms of dry gels were made on Kodak No-Screen



MUL.RB.MASTER

M	1	2	3
298	-1	91	42
299	82	89	52
300	75	98	41
301	-1	-1	-1
302	77	94	47
303	-1	-1	-1

MUL.RB.1

1	2	3	4	5	...
75	-1	96	70	86	...
76	72	115	94	95	...
77	74	126	96	102	...
78	56	91	68	74	...
79	59	-1	-1	-1	...
80	62	106	92	101	...
81	-1	-1	-1	-1	...
82	-1	-1	-1	-1	...

MUL.RB.2

1	2	3	4	5	...
88	79	-1	-1	85	...
89	88	70	-1	91	...
90	66	-1	77	72	...
91	78	-1	-1	80	...
92	83	79	76	81	...
93	80	72	78	82	...
94	86	73	-1	-1	...
95	87	74	82	96	...

MUL.RB.3

1	2	3	4	5	...
41	60	54	58	67	...
42	61	51	57	65	...
43	62	51	57	65	...
44	58	61	-1	64	...
45	56	50	54	-1	...
46	66	53	60	75	...
47	64	56	62	-1	...
48	57	55	-1	-1	...

FIG. 1. Individual proteins are followed through multiple gels and multiple experiments, using the mul.rb and mul.rb.master files. The contour numbers of the control film for each mul.rb table are located in column 1. The other columns list the contour numbers of matched spots from successive autoradiograms. A row holds a set of contour numbers belonging to a single polypeptide. The mul.rb.master table consists of the control columns (1, 2, and 3) from each mul.rb table and a master column (designated M). A "−1" signifies no record (spot not above threshold, not on film, or not resolved from another spot).

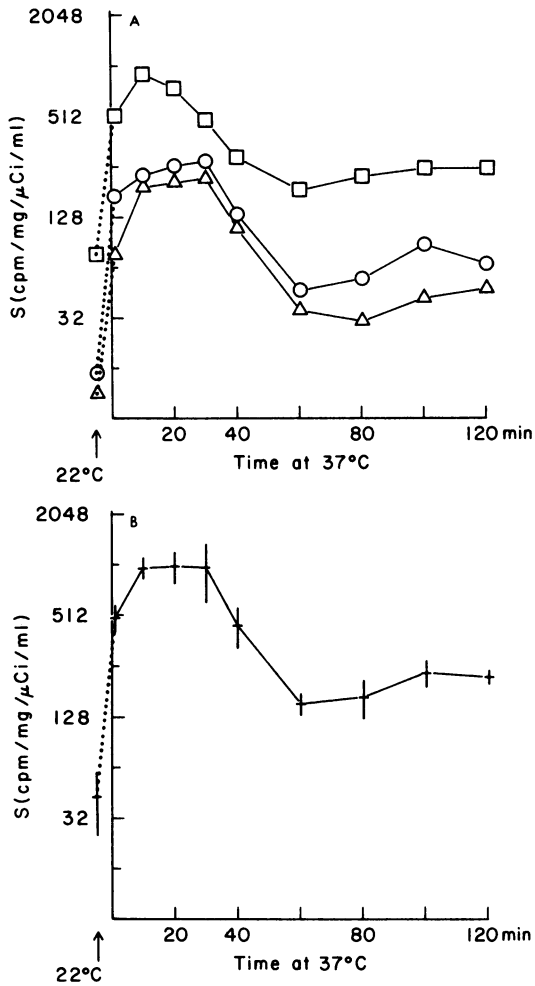


FIG. 2. Normalization and fusing of data. (A) Incorporation of L-[^{35}S]methionine into master spot 272 in three experiments. (B) Composite incorporation curve: data of experiments 2 and 3 are normalized to experiment 1 (using equation 3) and averaged. The error bars represent one standard deviation. Ordinate, Specific activity (SA); abscissa, starting time of 5-min labeling period.

or SB-5 film. The SB-5 film has only 80% of the speed of No-Screen and reaches saturation at a much lower density (absorbance = 2 versus 4 for the No-Screen). However, the SB-5 film has a finer grain size, a lower background, and a much shorter fixing time than the No-Screen (2 to 4 versus 8 to 18 min) and was, therefore, preferred. Films were developed by hand. The film density distributions of these autoradiograms were determined and analyzed on a computer-coupled film scanning system (7). Several further developments of the computational method have recently been described (42). In particular, one should draw attention to changes in the calibration method for translating film density in the autoradiogram (measured in terms of gray levels) to radioactive intensity in the gel (measured as counts per minute per square millimeter) in the method used to smooth the raw data coming from the film scanner, for splitting out overlapping contours belonging to adjacent spots, and for integration of radioactivity associated with individual proteins. Several additional computational methods, which have not yet been fully described, were used in analyzing these data. The new methods are described below.

Matching and normalizing data within a single experiment. The experiments described here consisted of many samples which were separately analyzed on two-dimensional gels. Experiments were also repeated. Thus, it became necessary to devise a reasonably convenient way of following the synthesis of many proteins through many different samples. This entailed paying close attention to how the data were organized and stored. As a gel was analyzed, each spot was assigned a unique number, specific to that gel. To follow an individual polypeptide through all of the autoradiograms generated in an experimental sequence, we made use of a summary file, mul.rb, the

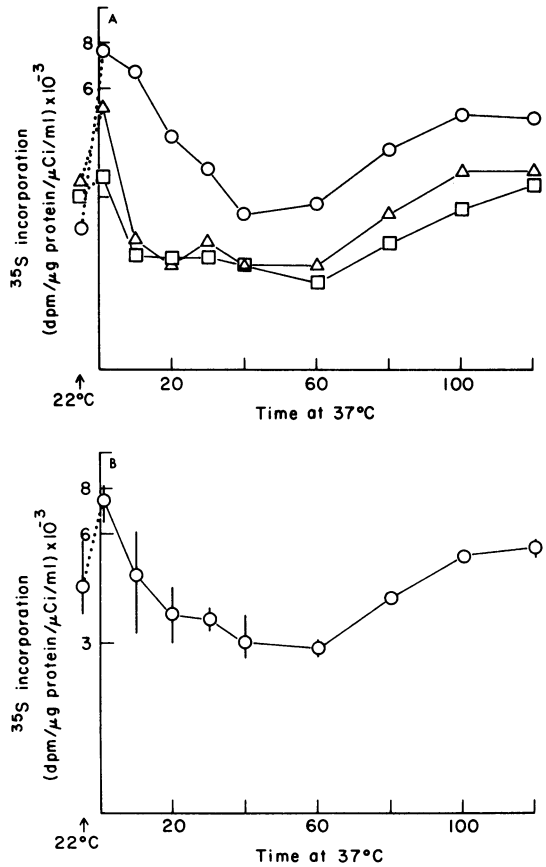


FIG. 3. (A) Incorporation of L-[^{35}S]methionine into total protein during the heat shock response. At time zero, cells were filtered and transferred to 37°C as described in the text. (B) Data normalized and fused as described in the text. Error bars represent one standard deviation. Abscissa, see legend to Fig. 2.

formation and format of which have been detailed elsewhere (42; see Fig. 1). In this file, the spot number, the total radioactivity (counts per minute per spot), and the maximum optical density (in terms of gray levels) are stored for each spot in each gel. Consequently, any spot in any gel is matched back to the designated control gel. (The first film in the time sequence comprising the datum set is usually designated as the control.) The total radioactivity in each spot was normalized for the counts per minute per milligram of total protein layered on the gel and for the microcuries of L-[^{35}S]methionine per milliliter in the labeling medium (that is, the normalized radioactivity is the number of counts per minute that would be found in a spot if the cells had been labeled in 1 μCi of ^{35}S per ml and if 1 mg of extracted protein had been layered on the gel) by means of a set of multiplication factors that are also maintained in the mul.rb file. There are, however, several parameters of electrophoresis and autoradiography that are not easily controlled. These included slight variations in the thickness of the gel resulting in variable quenching of the emitted particles, variable sensitivity of the X-ray film, variable penetration of protein into the isofocusing gel, and variable diffusion of protein out of the isofocusing gel during equilibration in preparation for the second dimension of electrophoresis. To compensate for these variations, we computed a normalization factor, F_k, based on the number of counts found in each film:

$$F_k = \frac{\sum_{i=1}^{q} C_{i1}}{\sum_{i=1}^{q} C_{ik}} \quad (1)$$

where C_{i1} and C_{ik} are the radioactivity associated with protein i in gel 1 (the control gel) and gel k, respectively, and q is the total number of proteins summed. The summation is carried over all spots matched between the two gels (these usually comprise at least 80% of the radioactivity generating each autoradiogram). Factor F_k varies on the average by about 10% from the ratio of radioactivities layered on the separate gels. Further normalization is made for variations of M_k, the quantity of protein (milligrams) layered on gel k, and of U_k, the quantity of L-[^{35}S]methionine (microcuries per milliliter) added to the medium (used in the range in which incorporation is proportional to the amount of added radioactivity). Combining all of the components gives the normalized radioactivity of spot i in gel k as:

$$S_{ik} = \frac{F_k}{M_k U_k} C_{ik} \quad (2)$$

In this paper, data on labeling of proteins are given in terms of S.

Matching and normalizing different exponents. To link together the results of several experiments, an additional file, mul.rb.master, was constructed to match the first (i.e., control) gels from each experiment. The organization of mul.rb. and of mul.rb.master is illustrated in Fig. 1. Here, each control gel (column 1 in mul.rb.1 to 3) was matched to a single master gel (M), which was generated from a very long exposure of a separate control gel. For example, master contour 302 was matched to control spots 77, 94, and 47 in experiments 1, 2, and 3, respectively; control spot 77 in experiment 1 was, in turn, matched to spots 74, 126, 96, and 102 in gels 2, 3, 4, and 5 of the same experiment, and control spot 47 was similarly matched to spots 64, 56, and 62 in gels 2, 3, and 4 of experiment 3. The designation −1 indicates that no matching spot is found (above threshold) in gel 5 of experiment 3. The file for the master autoradiogram is allowed to have many empty slots (that is, entries which provide lines that are blank in the control files). These are reserved for additional spots which are below the threshold of detection in the control gel, but which rise above threshold in one of the other gels of an experiment.

For the specific purpose of the experiments described here, we introduced an additional normalization. The need for this renormalization arose when the heat shock responses of individual proteins from separate experiments were compared (some were done a relatively long time apart). In these separate experiments, S_{i1} varied systematically, with variations

FIG. 4. The heat shock response domain. Three separate experiments were done. Each experiment comprised duplicate samples (numbered 1 and 2) of cells labeled at 22°C for 5 min and single samples (numbered 3 to 11) labeled for 5 min starting at each of the times after shift to 37°C that are shown on the abscissa of Fig. 3. Two-dimensional gels (pH 5 to 7 and 6 to 8) were prepared from the total solubilizable proteins of each sample. The correlation plots that are presented here involve only the proteins quantitated from autoradiograms of gels at pH 5 to 7. The fused data for approximately 350 proteins, which were matched throughout three experiments, are shown. The coordinates of each datum point are a pair of S values for a particular protein (i) in two sets of data that are being compared (see text for detailed definition of symbols). The following samples are correlated here. (a) Two scans were taken of a single autoradiogram of one of the 22°C samples, and each scan was separately analyzed. (b) Duplicate samples, labeled at 21 or 22°C (three pairs of samples from three separate experiments), were sorted into two comparison groups. Each comparison group contained one arbitrarily chosen sample from each experiment. (c) Comparison of proteins labeled 1 min after shift to 37°C with protein labeled at 21 or 22°C. (The match is between the fused and averaged data of three separate experiments.) (d) Comparison of proteins labeled 20 min after shift to 37°C with proteins labeled at 21 or 22°C (data base as in [c]). (e) Comparison of proteins labeled 120 min after shift to 37°C with proteins labeled at 21 or 22°C (data base as in [c]). (f) Comparison of proteins labeled 120 and 100 min after shift to 37°C (data base as in [c]). Symbols: +, spots that are below the threshold (b.t.) set for detection in the analysis or missing in one of the samples being compared; ●, major heat shock induction effect (in [c] and [d] only); □, major heat shock repression effect (in [c] and [d] only); all other spots are designated by X. Major heat shock proteins are defined as those whose rates of labeling are increased or decreased at least eightfold over the control (22°C) level at some point during the course of the experiment.

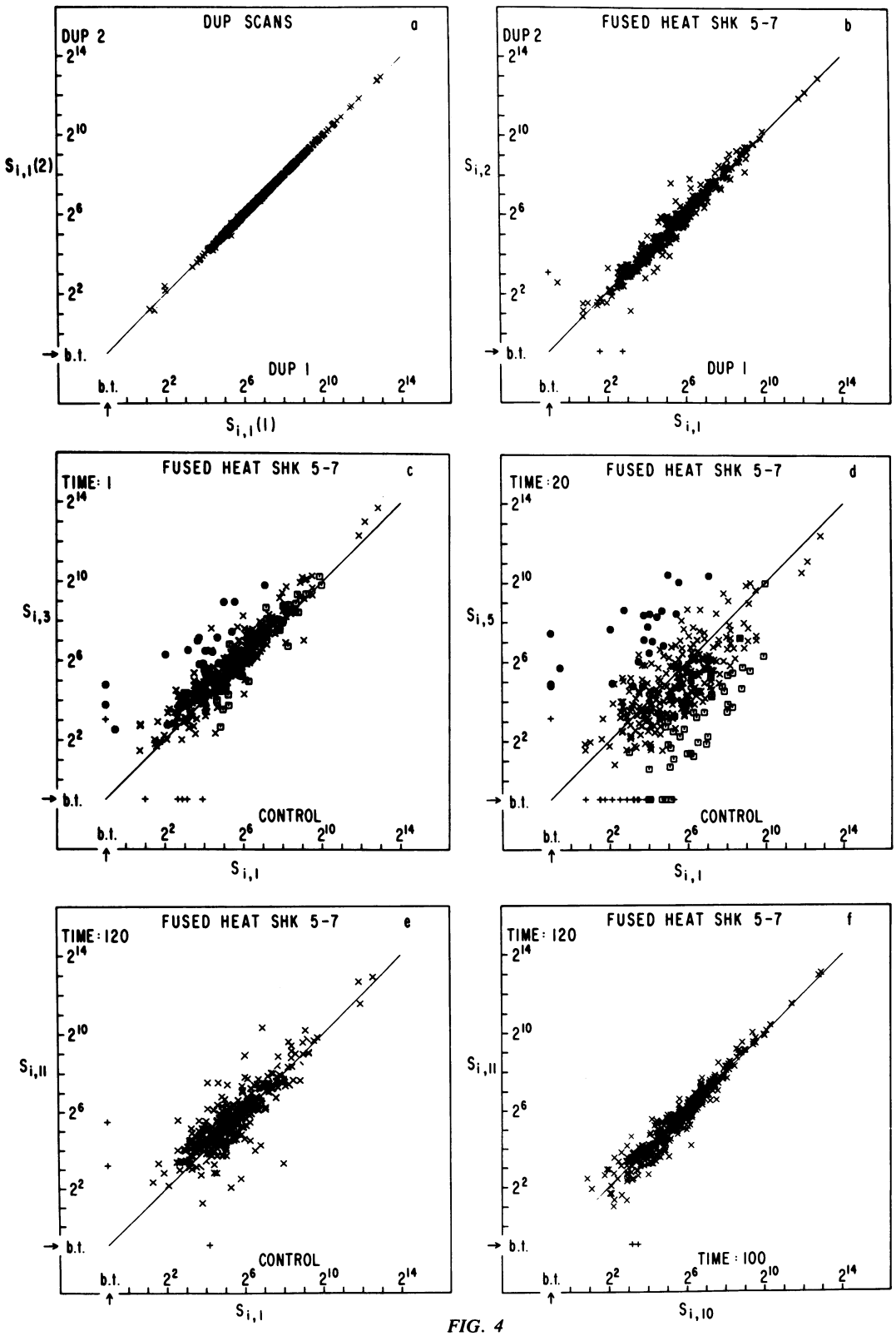


FIG. 4

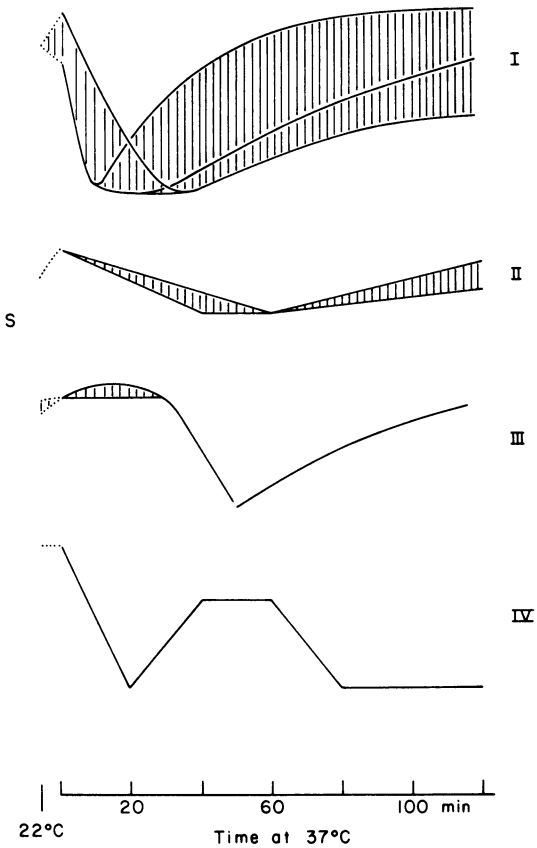


FIG. 5. Repression patterns. (I) The major category (which includes all major heat shock-repressed proteins). (II) More gradual repression than that of category I. (III) Repression onset delayed. (IV) Two cycles of repression.

of up to twofold not unusual, even for well-resolved and intense spots for which duplicate samples from the same experiment gave highly reproducible results. Thus, it was the pattern of incorporation into the same protein during different experiments which was relatively reproducible (Fig. 2). Since our primary interest was in the temporal pattern of incorporation, we found it useful to renormalize each set of data individually and to fuse the data into a composite graph. The renormalization factor, r_{im}, which is applied to all of the data for protein i in experiment m, is based on log S_{ik}, as follows:

$$r_i^{(m)} = \frac{\sum_k^N \log S_{ik}^{(1)}}{\sum_k^N \log S_{ik}^{(m)}} \qquad (3)$$

where $S_{ik}^{(m)}$ and $S_{ik}^{(1)}$ are the normalized radioactivities for protein i in gel k of experiments m and 1, respectively, and the sum is carried over N gels (total number of gels) in each set of data (experiment), but with N restricted to those datum points that are available for each experiment. For example, in the following made-up set of data (−1 denotes missing data; other numbers are values of $S_{ik}^{(m)}$).

Expt (m)	Gel(k)					
	1	2	3	4	5	6
1	100	120	90	70	50	70
2	50	70	55	40	−1	30
3	90	−1	80	40	45	55

Only k = 1, 3, 4, and 6 would be included in the summation for r_{i2} and r_{i3} (r_{i1} is, by definition, equal to 1). After $r_i^{(m)}$ was determined, the fused data (Fig. 2b) were calculated as:

$$\overline{\log S_{ik}} = \frac{1}{M}\sum_m r_i^{(m)} \log S_{ik}^{(m)} \qquad (4)$$

where the bar denotes the renormalization, and the sum is taken over the M experiments for which datum points S_{ik} are available (M is 3 in the above example). The above procedure amounts to taking geometric means (to lessen the dominance of the average by the largest values) and least squaring. The vertical bars in Fig. 2, 3, 6, 8–10, 14, and 15 represent standard deviations of S_{ik}.

RESULTS

Heat shock protein synthesis. *S. cerevisiae* M25 grew in L-tyrosine-supplemented MV-A minimal medium, with doubling times of 200 to 215 min at 22°C and 120 to 130 min at 37°C.

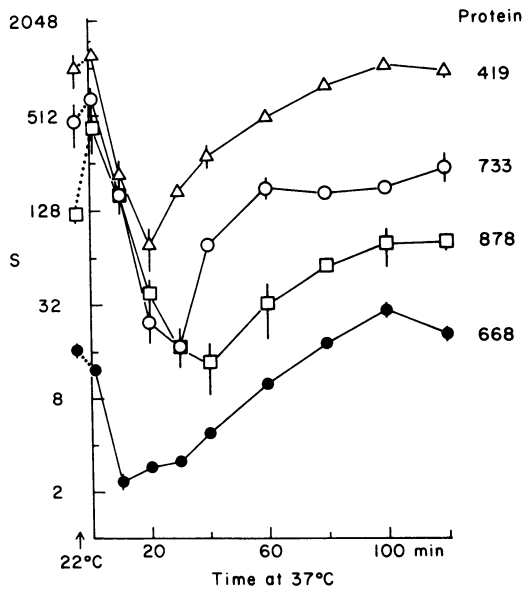


FIG. 6. Examples of repression category I. These four proteins were chosen to show diverse minima and different rates and extents of recovery. Their locations on the two-dimensional gels are shown in Fig. 12c.

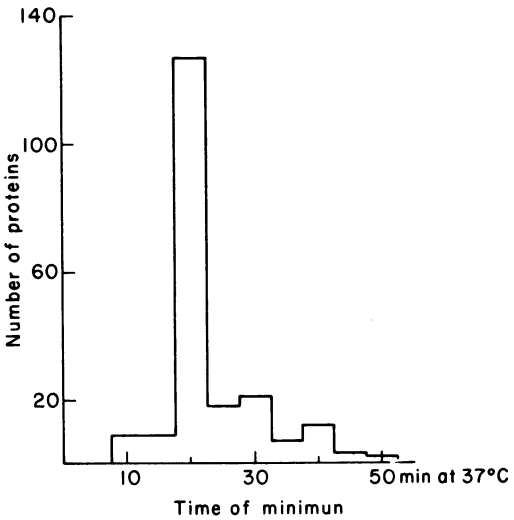


FIG. 7. Kinetic diversity within repression category I. The histogram shows the distribution of times, T_c, at which the minimum rate of synthesis of each protein is observed. A total of 209 proteins were sorted into this category. Proteins which were quantitated in pH 5 to 7 and pH 6 to 8 gels were counted only once.

When these cells were pulse-labeled with L-[^{35}S]methionine for 5 min at various times before and after shift from the lower to the higher temperature, the rate of incorporation varied (Fig. 3). Within 6 min after the shift to the higher temperature, the incorporation increased and then dropped rapidly to a minimum rate 40 to 60 min later and then gradually increased again. Figure 3 shows the appreciable range of values of three separate determinations with, however, always the same trends. (The variability primarily involved the immediate increase of incorporation after an increase of temperature and the subsequent drop.) The relatively minor temporal variation of global amino acid incorporation represents the summation of opposing effects in an extraordinarily pleiotropic response, in which the synthesis of certain proteins was transiently induced to various extents, that of many others was transiently repressed to various degrees, and that of still others was not greatly changed. Furthermore, the rates of synthesis of a small group of proteins were permanently changed (or at least remained distinctly different at the latest time after shift to the higher temperature that we examined). One effective way of representing the range of responses is through the scatter diagrams shown in Fig. 4.

The three experiments that are represented here consist of separate series of samples, labeled at 22°C and at various times after the shift to 37°C. Individual autoradiograms were analyzed (42), and then the data of the three experiments were combined and normalized as described above. The incorporation of ^{35}S into individual polypeptides was compared on log-log plots (to accommodate the great range of synthesis of different proteins). The first frame (Fig. 4a) shows that the reproducibility of quantitation of duplicate scans of an individual autoradiogram (Fig. 4a) or of duplicate autoradiograms (data not shown) is very high. Thus, for example, the standard deviation for the 514 spots in Fig. 4a was 1.5%. The variability of concurrently analyzed duplicate protein samples was much greater (Fig. 4b; standard deviation of 20% for 368 spots). Most of this variability was due to localized variations in the acrylamide gel, background noise, and interference from other, nearby spots. Large, low-intensity, streaky spots (i.e., those which do not focus well in the electrofocusing direction, a situation that is particularly prevalent among high-molecular-weight proteins) are relatively difficult to quantitate. Streaks and smeared spots also cause localized variations in the background which are not corrected for in the BACKGROUND program. At present, our spot detection programs make no attempt to resolve the density distributions of overlapping spots (1), and this is a source of systematic error and variability, particularly for

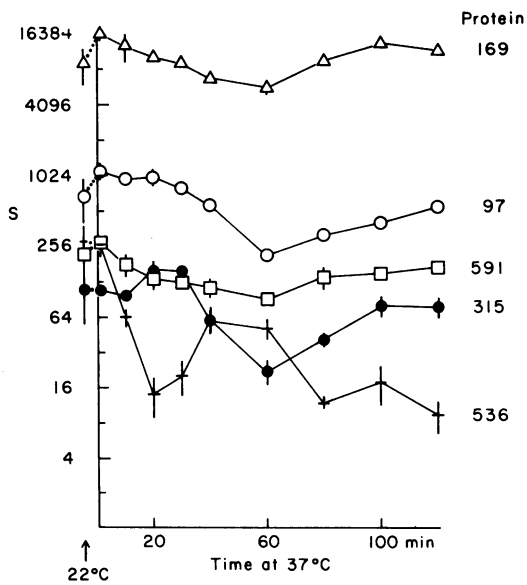


FIG. 8. Examples of repression categories II, III, and IV. The locations of these proteins on the two-dimensional gels are shown in Fig. 12 and 13. Symbols: △, protein 169 (category II; Fig. 13c); □, protein 591 (category II; Fig. 12c); ●, protein 315 (category III; Fig. 13c); ○, protein 97 (category III; Fig. 12c); and +, protein 536 (category IV; Fig. 12c).

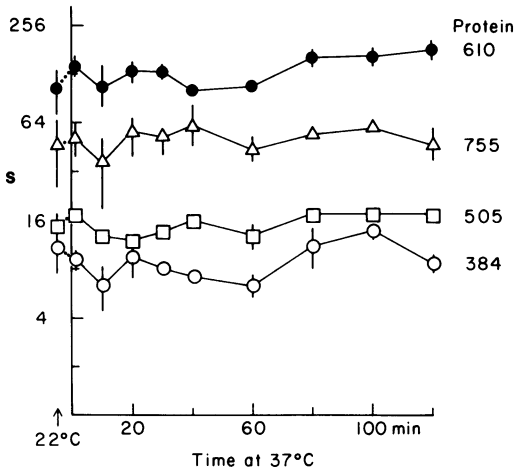


FIG. 9. Examples of proteins whose rates of synthesis do not undergo major changes as a result of a temperature shift (locations of these proteins on the autoradiograms are shown in Fig. 12c).

major spots that are close together and for small spots near large ones. In some instances, we observed irreproducible shifts in the locations of individual spots, relative to their neighbors, possibly due to variable postlysis protein modification. Nevertheless, the considerable sample-to-sample variation did not interfere appreciably with seeing the very diverse response of synthesis of individual proteins to the temperature shift. The spread of coordinates in Fig. 4c through f, relative to Fig. 4b, indicates the proportion of repressed and induced proteins as well as the range of effects. Dispersion was greatest at approximately 20 min after temperature shift (Fig. 4d) and then decreased (Fig. 4e), indicating that most protein synthesis is only transiently disturbed. The circles and squares in Fig. 4 indicate major heat shock proteins (the term "major" signifies that the rate of labeling is increased or decreased at least eightfold at some time after temperature increase). Many of the major induction proteins already exhibited their characteristic rise at the first labeling interval (1 to 6 min) after the temperature shift and are seen at the upper edge of the scatter diagram (Fig. 4c). The distribution of the circles in Fig. 4d is due in part to the fact that all of these major heat shock-induced proteins did not reach their maximum rates of synthesis at the same time. (We return to this question further on.) That residual, uncompensated changes of protein synthesis remained after 2 h at 37°C can be appreciated by comparing the scatter diagrams in Fig. 4e and 4f. The standard deviation in a comparison of samples labeled 100 and 120 min after shift up (Fig. 4f) was 17%, about the same as that for duplicate samples (Fig. 4b). On the other hand, a comparison of proteins labeled after 120 min at 37°C with proteins labeled at 22°C (Fig. 4e) showed that there are several "continuously" induced proteins; these are further discussed below.

Another way of looking at the heat shock response in Fig. 4 is to examine the unmatched entries along the dotted lines parallel to the ordinate and abscissa (+; axes marked b.t. for below threshold). These entries represent spots that were detected and quantitated in one film of a pair but not in the other. For minor proteins, these entries primarily represent maximum film densities that lay above and below the chosen threshold in the two films that were being compared. (Also, proteins which focus at the very edges may be resolved in one gel, but not in the other; such immaterial instances have been edited out of the data.) There are only three b.t. entries on the abscissa and ordinate in Fig. 4a, all corresponding to minor proteins near the limit of detection. The number of entries along the abscissas and ordinates increased in Fig. 4c and 4d and then decreased again in Fig. 4e. These entries also reflect the transient heat shock response. By and large, they do not indicate absolute induction or repression of synthesis of individual proteins. For example, examination of the quantitative data and of the autoradiogram shows that for the five entries along the ordinate in Fig. 4d, labeling of the corresponding proteins at 22°C was visually detectable in heavy exposures.

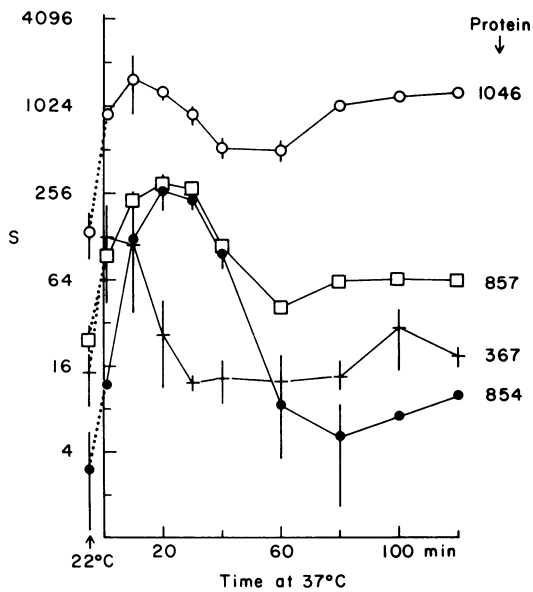


FIG. 10. Examples of proteins which are induced after shift to 37°C (locations of these proteins on the autoradiograms are shown in Fig. 12c).

Temporal patterns of repression. We next turn to a more detailed presentation of our analyses of the transient heat shock response, a presentation that is made more complex by the fact that the response is highly noncoordinate. Fortunately, there are certain dominant patterns that allow elements of generalization to emerge. With regard to proteins whose synthesis is transiently repressed after shift from 22 to 37°C, one major and several minor patterns can be discerned (Fig. 5). As will become plain, the limits of these stereotypes are arbitrarily conceived within an essentially continuous variation, but the suggested classification is at least useful in allowing reasonably brief communication of the detailed data.

(i) Category I (Fig. 5 and 6). Category I is the major pattern of response. In most (but not all, see protein 668) instances, incorporation of L-[^{35}S]methionine into the protein rose immediately after shift to 37°C and then decreased relatively sharply to less than the 22°C rate and recovered at various rates, but not necessarily to the 22°C level. The specific examples in Fig. 6 show various extents of repression, various rates of recovery, and various times at which the minimum rate of protein synthesis is displayed. Over 200 protein spots were sorted into this category (out of over 500), including all of the proteins which undergo the most severe repression. Predominantly, the proteins of category I had their lowest rates of protein synthesis 20 to 25 min after shift to the higher temperature (Fig. 7), and much of the recovery occurred by 60 min (Fig. 6).

(ii) Category II (Fig. 5 and 8). A much smaller group of proteins (ca. 20) was sorted into a category marked by more gradual and less extensive repression. Five of the six most abundant proteins (which together made up almost 30% of the total protein resolved in the pH 5 to 7 and 6 to 8 gels) fell into this category. Thus, it is not surprising that the temporal pattern of synthesis in this group most closely resembles the pattern of global [^{35}S]methionine incorporation. Two specific examples are shown in Fig. 8 (curves 1 and 2). It should be appreciated that categories I and II are contiguous and that some assignments are ambiguous; the narrow distribution of the characteristic time in Fig. 7 results in part from having separated category II from category I. On the other hand, proteins 419 (Fig. 6) and 169 (Fig. 8, curve 1), which exemplify recurrent properties, clearly are not coordinately expressed.

One other point about proteins 169 and 591 is worth emphasizing. The time dependence of S_i is very close to the time dependence of total L-[^{35}S]methionine incorporation (shown in Fig. 3b). Thus, if S_i were to be normalized for that

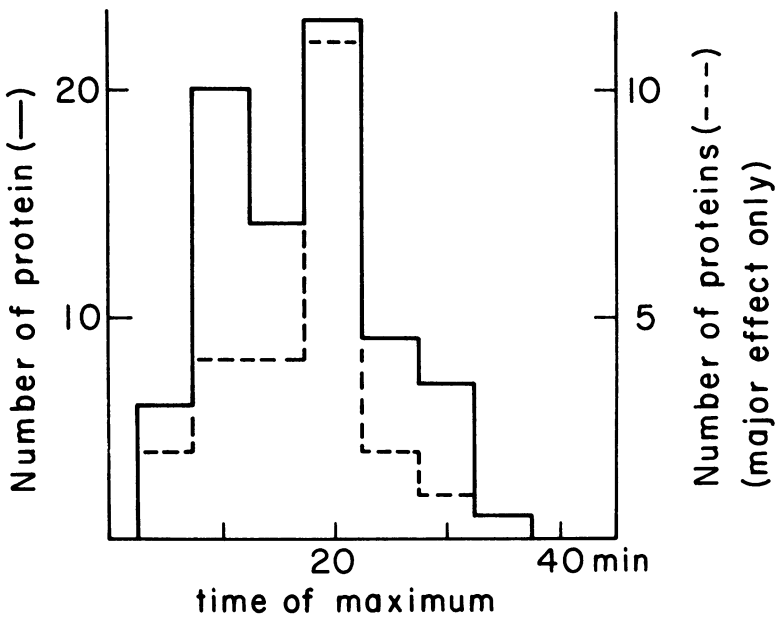


FIG. 11. Kinetic diversity among the heat shock-induced proteins. Abscissa, Time at which the maximum rate of labeling is observed; ordinates, numbers of proteins. Heavy curve (and left-hand scale), 80 proteins identified on gels (pH 6 to 8 and 5 to 7). Dashed curve (and right-hand scale), 24 proteins which show the greatest heat shock induction effects (maximum rate of labeling at 37°C reaches at least eight times the 22°C rate). The map locations of these proteins are shown in Fig. 12b and 13b.

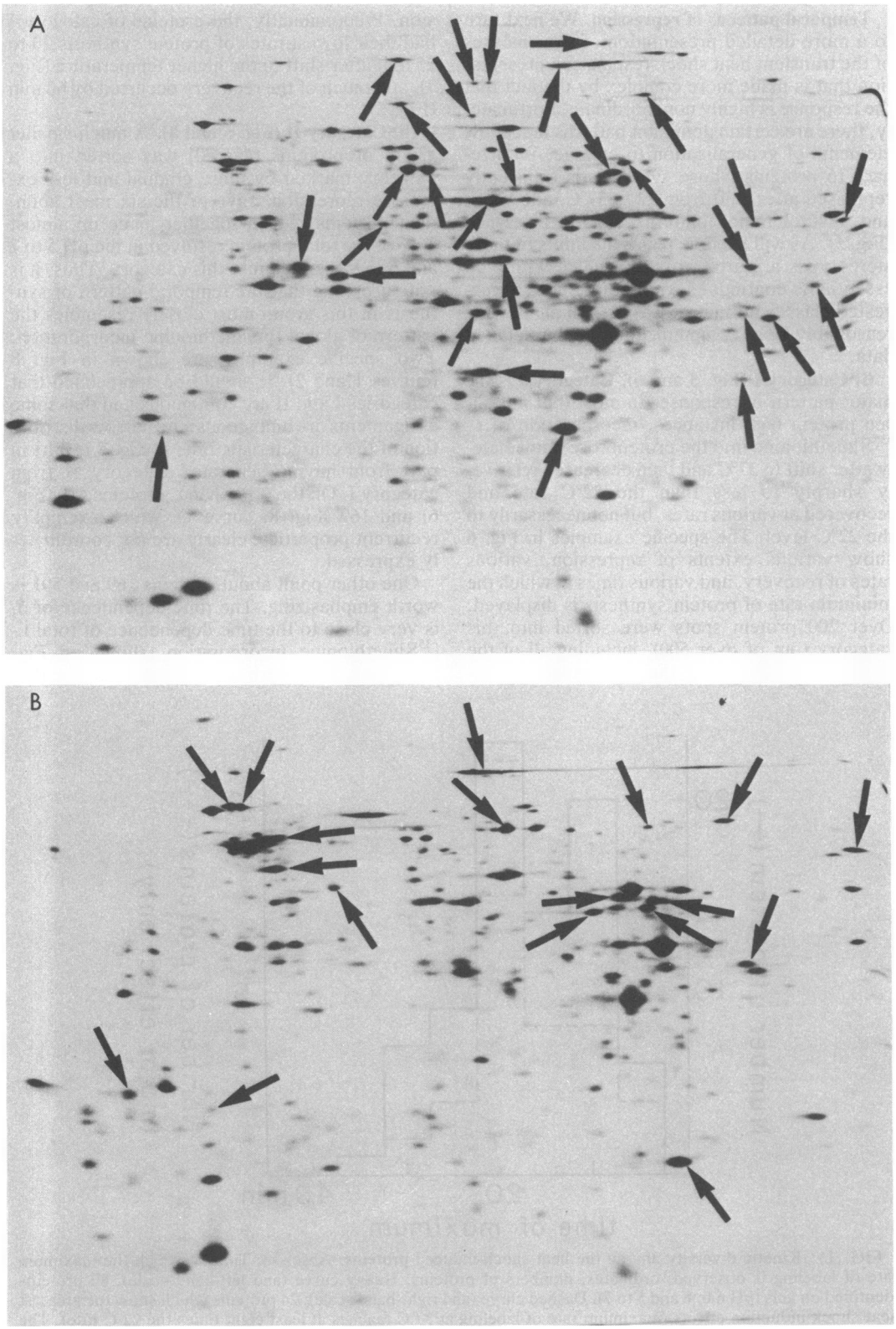


FIG. 12A–12B

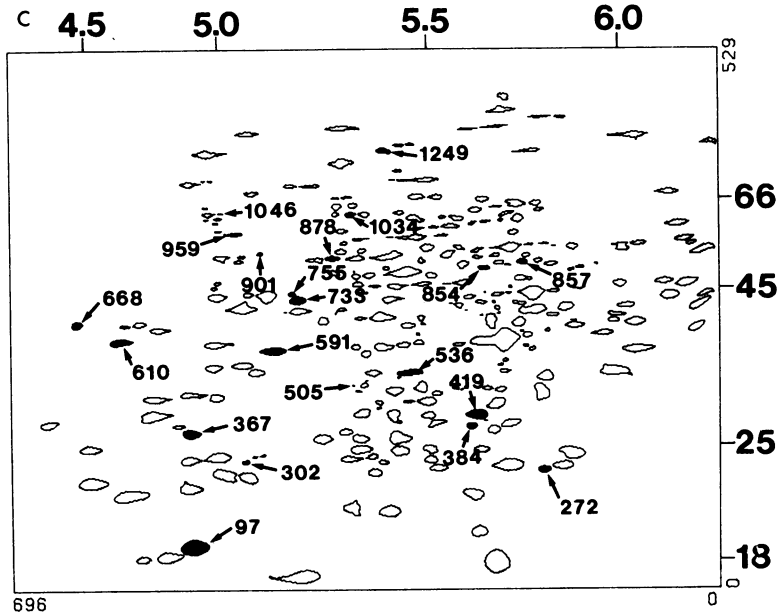


FIG. 12. Protein maps (pH 5 to 7 range). (A) Autoradiogram of resolved proteins isolated from cells grown and labeled at 22°C. Arrows indicate those polypeptides whose synthesis undergoes a major degree of repression (defined in the text) during the course of the temperature shift. (B) Proteins labeled 20 min after being shifted to 37°C. Arrows indicate proteins whose synthesis undergoes major stimulation during the course of the temperature shift. (C) Computer-generated contour map of the autoradiogram in (A). All of the protein spots mentioned in the text are indicated by arrows and numbers. The right-hand vertical scale indicates the approximate molecular weight (in kilodaltons), and the horizontal scale represents the approximate apparent pH range.

variation of total incorporation, proteins 169 and 591 would be regarded as virtually invariant (see below). However, such a normalization is not necessarily proper. It would be appropriate if the temperature shift to 37°C were to directly affect the general, total capacity of the cell for protein synthesis, according to the time course shown in Fig. 3b. But that cannot be entirely correct, since Fig. 3b is also resultant of all of the heat shock induction and repression effects on all of the proteins of the cell.

(iii) Category III (Fig. 5 and 8). A very small number of proteins showed a significantly delayed onset of repression. Examples of two of the six spots that were sorted into this category are shown in Fig. 8 (proteins 97 and 315). We have assured ourselves that, within the resolution limits of the electropherograms, the spots in this category do not appear to be composite. The type III pattern could be generated by temperature shock-dependent induction and repression mechanisms operating on a single protein, with somewhat different time parameters.

(iv) Category IV (Fig. 5 and 8). A very small number of proteins showed two successive cycles of repression. One particularly clear example is shown in Fig. 8 (protein 536). Approximately 10 others also underwent two cycles of repression, but with smaller ranges of effect.

We arbitrarily define major heat shock repression effects in terms of a minimum rate of synthesis at 37°C which is less than one-eighth of the rate at 22°C in at least two of the three experiments. The effects in this category are indicated by squares in the scatter diagram (Fig. 4), and the corresponding proteins are identified with regard to molecular weight and apparent isoelectric point (see Fig. 12 and 13). Including pH ranges, 42 proteins were subject to major heat shock repression effects. As already stated, the repression patterns for these proteins all fall into category I.

The rates of incorporation of L-[^{35}S]methionine into a large number of proteins (ca. 25% of those analyzed) changed relatively little during the heat shock transient; some examples are shown in Fig. 9. That even these proteins underwent minor variations in response to the temperature shift can be seen by comparing appropriate correlation plots (data not shown). Indeed, with respect to magnitude of effect (but not with respect to temporal pattern), this category of proteins is contiguous with repression category II.

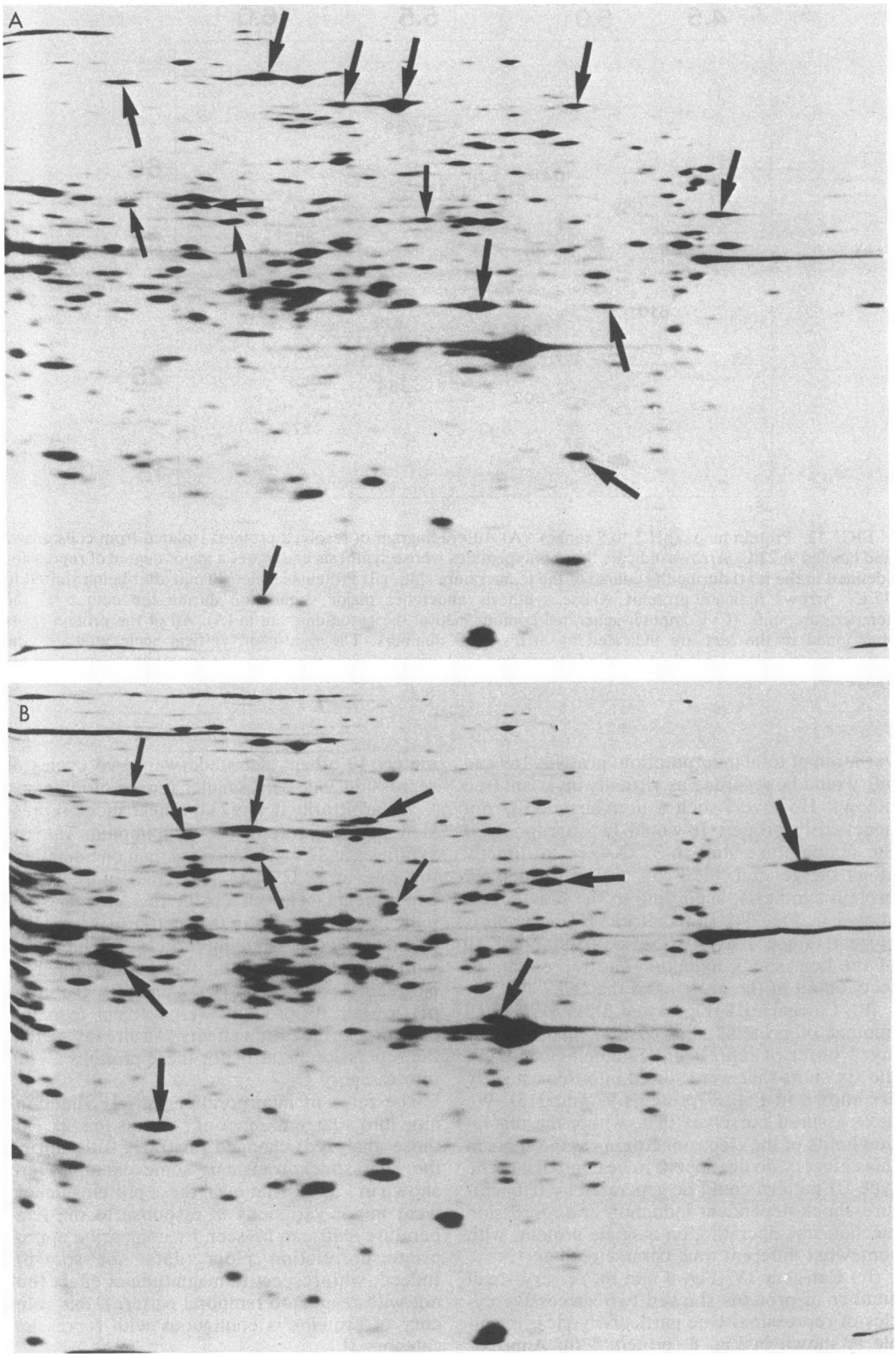


FIG. 13A–13B

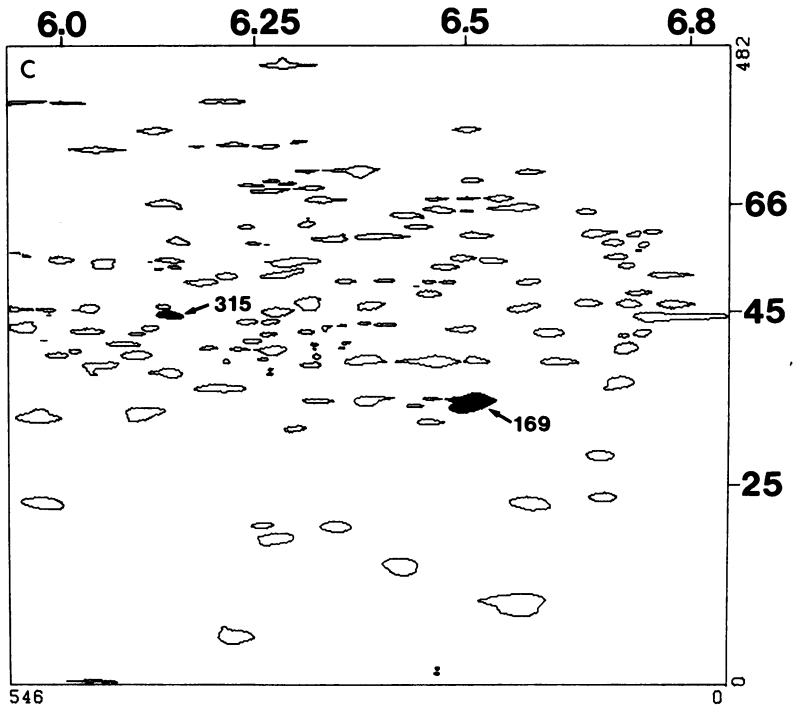


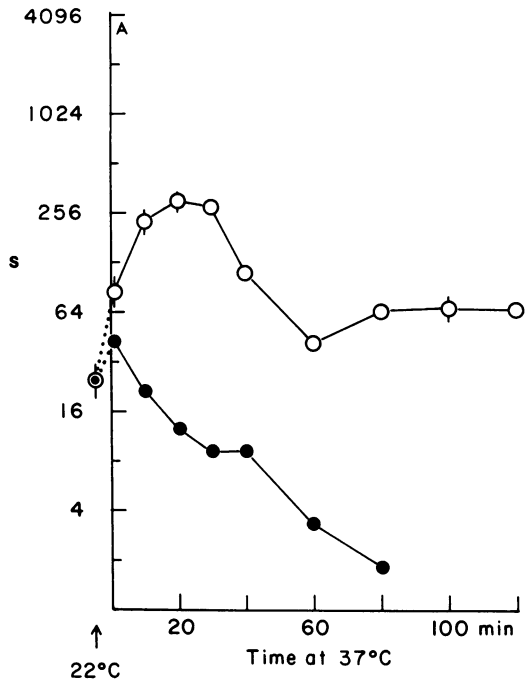
FIG. 13. Protein maps (pH 6 to 8 range). For explanation, see legend to Fig. 12.

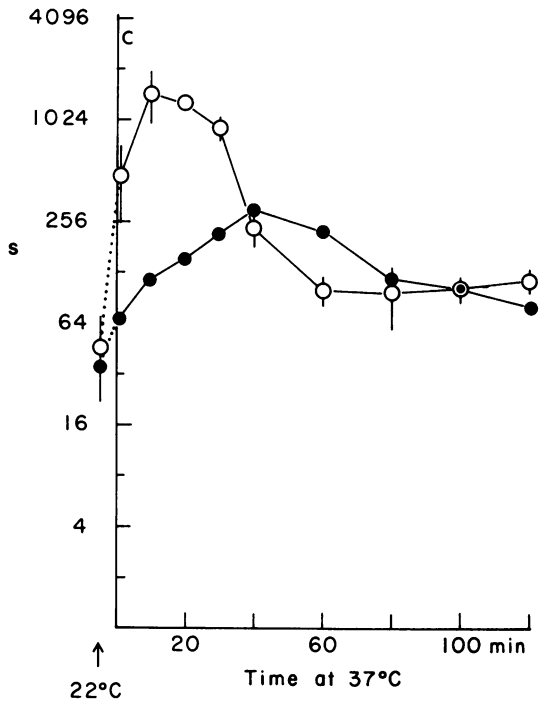
The preceding comments about compensating S_i of category II proteins for changes of total incorporation of radioactivity also apply to these proteins. Consequently, there is some ambiguity about which, precisely, are the constant proteins. We have decided against introducing a compensation for total incorporation because that would merely shift, rather than resolve, the ambiguity about which, precisely, are the constant proteins.

Temporal patterns of induction. The sharp temperature increase from 22 to 37°C elicited an increase in the rate of synthesis of many yeast proteins (Fig. 10). For many of these heat shock-induced proteins, incorporation of ^{35}S increased immediately, reached a maximum 20 to 30 min after the temperature shift (e.g., proteins 854 and 857), returned to approximately the 23°C rate, and then possibly increased again slightly. The local minimum (at 40 to 60 min after shift to 37°C) and subsequent slight rise appear to correlate with the gradual increase of global amino acid incorporation late after temperature shift (Fig. 3). For a small number of proteins (e.g., 367 in Fig. 10) the rate of protein synthesis rose immediately after temperature shift, but stayed at the elevated level for only a brief period. For another small group, including protein 1046, the rate of protein synthesis increased rapidly after temperature shift and stayed relatively high for the entire duration of the experiment. This small group might include temperature-indicating proteins.

A histogram showing the times of maximum synthetic rate of the heat shock-induced proteins is presented in Fig. 11. Those proteins that underwent the greatest degree of induction show a rather narrower range of temporal variation consistent with the possibility that most of these might be coordinately regulated. The major heat shock-induced proteins (i.e., those whose maximum synthetic rates after temperature shift reached at least eight times the 22°C rate) are mapped in Fig. 12 and 13. Excluding overlap, there are at least 28 such proteins in the gels at pH 5 through 7 and 6 through 8.

Effects of blocking mRNA production. The temperature shift experiments were repeated with strains M304 and M421, which are homozygous for nonallelic, temperature-sensitive mutations in mRNA production, the first (*rna1* in M304) affecting mRNA transport to the cytoplasm and some RNA processing (14, 36) and the other (in M421) affecting RNA synthesis (39). The *rna1* mutation abolishes the induction of allophanate hydrolase at 37°C (5, 6) and eventually reduces the synthesis of most yeast proteins to very low levels (e.g., see Fig. 15a; Miller et al., unpublished data). As expected (28, 30), the induction of most heat shock proteins is





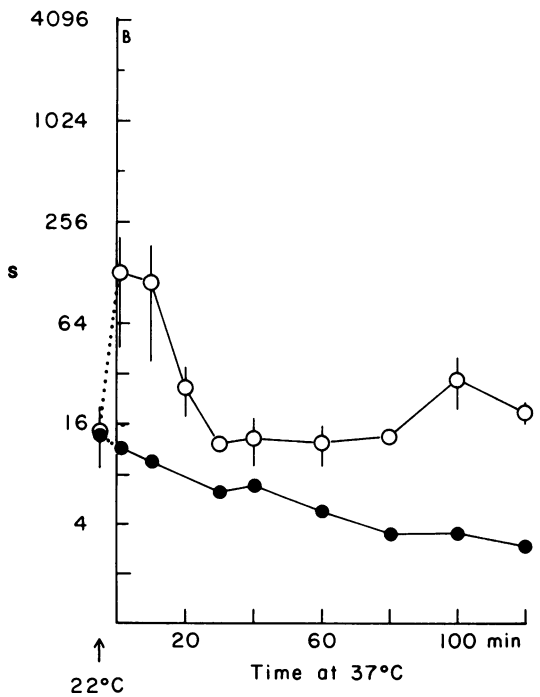
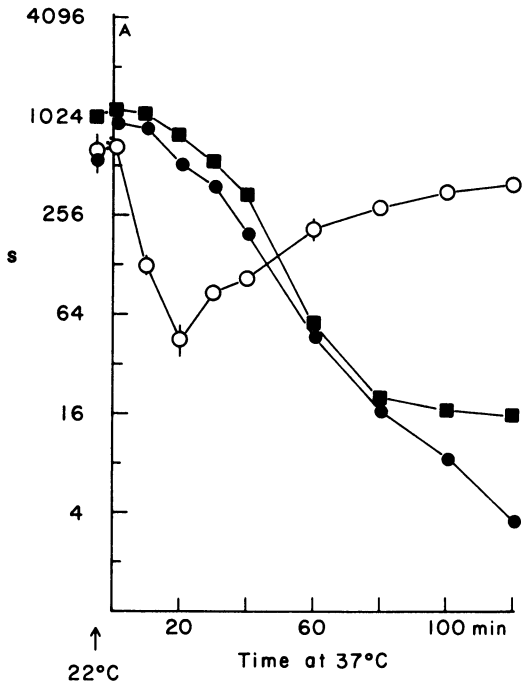


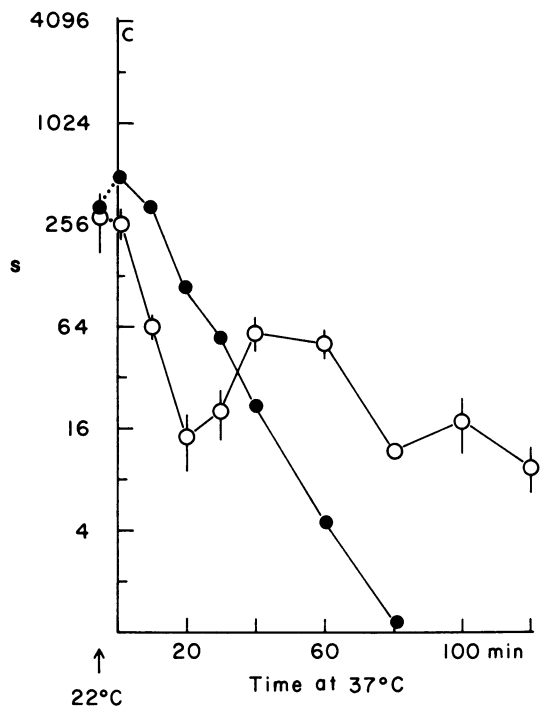
FIG. 14. Induction of synthesis of heat shock proteins at 37°C in wild-type and RNA metabolism-defective cells. (A) Protein 857, (B) protein 367, (C) protein 1249. Symbols: ○, wild type (also see legend to Fig. 10 for proteins 857 and 367); ●, strain M304; ■, strain M421. Vertical bars are standard deviations.

blocked at 37°C in these mutants (e.g., protein 857, Fig. 14a), even those that show very rapid induction (e.g., protein 367, Fig. 14b). However, Fig. 14c shows an example of a heat shock protein (protein 1249) whose synthesis is significantly stimulated in the absence of new RNA synthesis. At least for this protein, there appears to be a significant nontranscriptional component to the heat shock induction. The restoration of the steady rate of synthesis of protein 1249 in wild-type cells at 37°C (after the induction burst) is also quite rapid in view of the general stability of this message in strain M304 at 37°C. It should be borne in mind that, in the RNA synthesis mutant, one is looking at a cell which is ultimately almost completely depleted of its mRNA. The wild-type cell, which is recovering from the heat shock transient, is making protein at nearly the normal total rate (Fig. 3b). Functional and chemical stabilities of mRNA under such different circumstances are not necessarily the same.

We have already noted that a few proteins (e.g., protein 959) are continuously synthesized at 37°C. This does require de novo RNA synthesis (data not shown).

We previously reported (30) that the repression of synthesis of some proteins at 37°C is very rapid, relative to the decay of protein synthesis in the absence of RNA synthesis. Another example is shown in Fig. 15a (protein 1034). This is not the case for proteins in repression categories II and III (Fig. 15b). Here, it appears that there





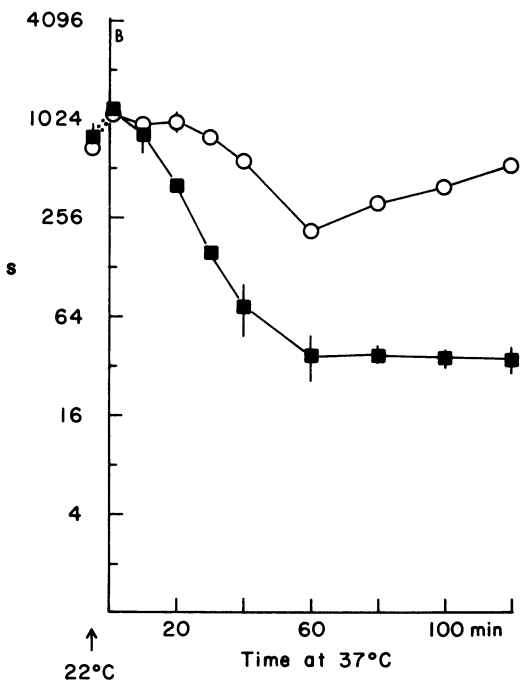


FIG. 15. Repression and recovery of synthesis of proteins at 37°C in wild-type and RNA metabolism-defective cells. (A) Protein 1034 (category I), (B) protein 97 (category III), (C) protein 536 (category IV). Symbols, see legend to Fig. 14.

must be substantial residual transcription at 37°C during the heat shock transient. The complex category IV kinetics is dependent on continued delivery of mRNA to the cytoplasm (protein 536, Fig. 15c).

DISCUSSION

The analysis which has been presented here displays the highly pleiotropic nature of the yeast heat shock response. In yeast, as in other organisms, transient exposure to high but tolerable temperature protects against rapid killing at otherwise lethal temperatures. We have chosen 37°C as the temperature for eliciting the heat shock response. Although our wild-type *S. cerevisiae* cells grow indefinitely at this temperature, it is known that 37°C is very close to the limiting temperature, above which growth is accompanied by at least some thermal cell death (41). Certainly, it is not necessary to go to this superoptimal temperature range to elicit at least some protection from thermal killing (data not shown).

The synthesis of more than 80 of the approximately 500 proteins that were quantitatively analyzed in these experiments was induced at 37°C (Fig. 11). About 20 of these are regarded as major heat shock proteins (defined as those whose synthesis is induced at least eightfold at some time during the response) (Fig. 11b). If all of those proteins have a role in protecting cells from heat death, they might well be guarding

many different targets. The synthesis of many other proteins is, to some degree, transiently repressed at 37°C. Several general patterns of repression have been distinguished, the most common involving a rapidly decreasing rate of synthesis, reaching a minimum 10 to 30 min after the temperature shift, followed by more-or-less rapid recovery to about the preshift level.

It is now established that in *Drosophila* spp., for example, the heat shock response involves new transcription and alterations of translational selectivity. The mRNA that becomes untranslatable in vivo as a consequence of the heat shock response is not degraded and can be extracted in (in vitro) active form. For yeasts, the picture appears to be different. Repression of synthesis of certain prominent proteins correlates with loss of the conjugate mRNA (23, 27). However, those experiments lacked the (protein) resolution of which the current experiments are capable. We tried, therefore, to identify proteins that might be regulated in different ways. Instead of examining in vitro translation products, we looked at the effects of mutations affecting RNA synthesis and transport to the cytoplasm (30; Miller et al., manuscript in preparation). We had previously shown that the *rna1* mutation, which blocks transport of RNA to the cytoplasm and processing of certain RNA species at 37°C (14, 36), blocks both the induction and the rapid repression of many heat shock-affected proteins (27, 30). In the current survey of heat shock responses (Fig. 10), we noted two heat shock-induced proteins whose maximum rates of synthesis occurred almost immediately after induction. Even these very rapid inductions are blocked by the *rna1* mutation (Fig. 14b) and by a temperature-sensitive RNA synthesis mutation (39; Miller et al., manuscript in preparation).

On the other hand, at least three of the major heat shock proteins show significant increases of synthetic rate after shift to 37°C in the two temperature-sensitive mutants which affect mRNA production in entirely different ways (Fig. 14c). The stimulation of protein 1249 can also be seen in a petite derivative of strain M304 (data not shown). Thus, it seems clear that this protein is the product of a nuclear gene and that there is a post-transcriptional component to the stimulation of protein synthesis.

The repression of synthesis of certain category I proteins after shift to 37°C is so rapid that it must occur, at least in part, at the post-transcriptional level (since it cannot be accounted for by mRNA decay at 37°C after cessation of transcription [30]). Not all category I proteins are subject to this very rapid repression. For example, protein 878 (Fig. 6) is not (data not shown). Those category II repression proteins which we have examined are also not subject to more rapid repression than their normal mRNA life times (in the absence of heat shock) would indicate (Fig. 15b). For proteins such as 610 or 755 (Fig. 9), which are not substantially affected by temperature up-shift, synthesis of the conjugate mRNA must continue when wild-type cells are shifted to 37°C (although not necessarily at the same rate as at 22°C (data not shown), because experiments with the mRNA production mutants (e.g., Fig. 15b) and with lomofungin (data not presented) show that the mRNA is not stable.

In closing, it is appropriate to stress, once again, that the *S. cerevisiae* heat shock response is diverse. Most probably, there is a core of coordinately regulated and affected genes which are turned on and off together, with roughly similar kinetics. Many yeast proteins are unaffected by shift to 37°C; yet, others are affected, but with such different kinetic properties that different or additional regulatory mechanisms must be involved.

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