17. P. Bochsler [in *Solar Wind Five*, M. Neugebauer, Ed., *NASA CP-2280* (1983), p. 613] has discussed the broadening of charge state spectra resulting from mixing ion populations originating at different temperature.
18. Freeze-in temperatures are calculated here by assuming Maxwellian distribution functions for the electrons. Distorted distribution functions (κ distributions) would lead to even lower freeze-in temperatures [A. Bürgi, *J. Geophys. Res. A* **92**, 1057 (1987)].
19. Theoretical studies and modeling have lead many authors to discuss plasma waves as an important momentum source for driving HSSTs; see R. H. Munro and B. V. Jackson, *Astrophys. J.* **213**, 874 (1977); J. V. Hollweg, *Rev. Geophys.* **16**, 689 (1978); *J. Geophys. Res.* **91**, 4111 (1986); J. F. McKenzie, W.-H. Ip, W. I. Axford, *Astrophys. Space Sci.* **64**, 183 (1979); R. Lallement, T. E. Holzer, R. H. Munro, *J. Geophys. Res.* **91**, 6751 (1986); (*15*).
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22. One solar rotation (days 308 through 334 of 1992) was omitted because a coronal mass ejection occurred, so that our analysis includes 9.5 solar rotations.
23. The data for V_α and T_O are the same as those shown earlier (*9*), whereas that for T_C and Fe/O are new. For Mg/O, we used the charge spectrum Mg^{6+} to Mg^{10+} for determining the Mg abundance, which should reduce systematic errors, as compared to our earlier publication (*9*), where we used the main ion Mg^{10+} and estimated the abundances of the other Mg ions by assuming identical freeze-in temperatures for Mg and O.
24. We are very grateful to the teams of engineers and physicists in our institutions as well as the project teams of the European Space Technology Center and the European Space Operations Center of the European Space Agency and the Jet Propulsion Laboratory (JPL) of the National Aeronautics and Space Administration (NASA) for their decisive contributions to the success of SWICS and the Ulysses mission. We are indebted to P. Bochsler and M. C. E. Huber for valuable discussions. This work was supported by the Swiss National Science Foundation, NASA-JPL contract 955460, and the Minister für Forschung und Technologie of Germany.

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Requirement of a Small Cytoplasmic RNA for the Establishment of Thermotolerance

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Thermotolerance is an inducible state that endows cells with an enhanced resistance to thermal killing. Heat shock proteins are believed, and in a few instances have been shown, to be the agents conferring this resistance. The role of a small cytoplasmic RNA (G8 RNA) in developing thermotolerance in *Tetrahymena thermophila* was investigated by creating a strain devoid of all functional G8 genes. These G8 null cells mounted an apparently normal heat shock response, but they were unable to establish thermotolerance.

A strong positive correlation between the accumulation of heat shock proteins (hsps) and the ability of cells to maintain viability at or above normally lethal temperatures has been noted for a number of years (*1*). However, recently it has been shown that specific stress-induced proteins are absolutely required for the establishment of thermotolerance (*2*).

We previously showed that, in response to heat shock, starvation, or entry into the stationary growth phase, the ciliated protozoan *Tetrahymena thermophila* rapidly accumulates a small cytoplasmic RNA (approximately 300 nucleotides) called G8 that quantitatively associates with ribosomes (*3–5*). Unlike other heat-inducible genes, the gene encoding G8 RNA is transcribed by RNA polymerase III (*3*). The kinetics of accumulation of G8 RNA on ribosomes coincides with changes in the fractions of hsp and non-hsp mRNAs translated during heat shock (*3, 6, 7*). G8 RNA shares weak homology with 7SL and 4.5S RNA (*4*), two RNAs known to affect ribosome function (*8*). Furthermore, as revealed by Northern (RNA) blot analysis, G8 RNA forms a stable duplex with the large (28S) but not the small (18S) ribosomal RNA; antisense G8 RNA hybridizes to neither (*9*). We therefore proposed that G8 RNA might be part of a machinery that regulates selective translation of different classes of mRNA during stress situations (*4*).

To test this hypothesis, we used genetic manipulation methods (*10–12*) to create a strain of *T. thermophila* in which the approximately 50 macronuclear copies of the gene encoding G8 RNA were inactivated by insertion of a neomycin (*neo*) gene (Fig. 1A) (*13*). Expression of the *neo* gene confers on *T. thermophila* an increased resistance to paromomycin (*12*). Although initial transformants contained copies of both the functional and the inactivated G8 gene, the continued growth of such cells in increasingly higher concentrations of paromomycin eventually selected for cells that contained only the disrupted form of the gene (Fig. 1B). G8 null cells did not produce G8 RNA when starved or when exposed to a 39°C heat shock for 2 hours (Fig. 1, C and D), conditions that normally enhance expression of G8 RNA (*3, 4*). These results indicated that the G8 gene is not essential for normal vegetative growth.

To investigate whether G8 RNA is required during heat shock, we transferred early logarithmic phase wild-type and G8 null cells from 30°C to 39°C and maintained them at 39°C for 24 hours. Under these conditions, wild-type cells initially stop growing and synthesize predominantly hsps for about 1.5 hours (*7, 14, 15*). As the synthesis of non-hsps returns, growth recommences, albeit at a reduced rate (*14*). The synthesis of hsps and the return to non-hsp synthesis in G8 null cells appeared almost normal, although the return of non-

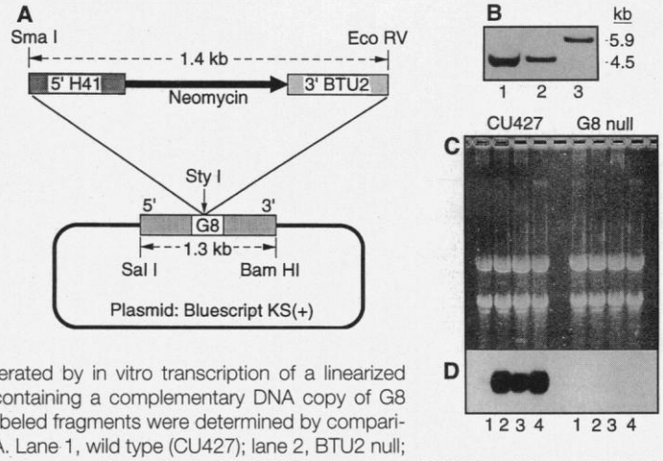

Fig. 1. Generation of a G8 deletion strain. (**A**) Construction of a G8 gene disrupted with a neomycin gene (*13*). (**B**) Southern (DNA) blot analysis of strains with and without disrupted copies of the G8 gene. DNA was cut with Eco RI, subjected to electrophoresis on a 0.8% agarose gel, transferred to a filter, and probed with a ^{32}P-labeled antisense copy of the G8 gene generated by in vitro transcription of a linearized Bluescript plasmid (pBS) containing a complementary DNA copy of G8 RNA (*3*). The sizes of the labeled fragments were determined by comparison with Hind III–cut λ DNA. Lane 1, wild type (CU427); lane 2, BTU2 null; and lane 3, G8 null. (**C** and **D**) Northern blot analysis of G8 RNA isolated from CU427 or G8 null cells. Total RNA was isolated as in (*4*). Lanes 1, cells in early log phase at 30°C; lanes 2, cells shifted to 39°C for 2 hours; lanes 3, cells transferred to starvation medium for 2 hours; and lanes 4, cells in starvation medium for 5 hours. The gels were stained with ethidium bromide (C), and the RNA was then transferred to a filter that was hybridized to a ^{32}P-labeled antisense copy of G8 RNA and autoradiographed (D).

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hsp synthesis appeared somewhat diminished in the G8 null cells (Fig. 2, A and B). Nevertheless, as with wild-type cells, the G8 null cells recommenced growth at 39°C. We conclude that G8 RNA is not absolutely required for the translation of hsp mRNAs during heat shock or for the translation of non-hsp mRNAs during or after recovery from heat shock.

To determine if the absence of G8 RNA compromised the viability of starved cells in any way, we washed G8 null cells into starvation medium at 30°C and, after various incubation periods, measured their viabilities. Even after 2 days, no differences in colony-forming ability were found between G8 null and control cells (Fig. 2C). However, when cells in starvation medium were incubated at 39°C (an hsp-inducing condition) for various lengths of time and then returned to growth medium at 30°C, G8 null cells more rapidly lost viability as compared with wild-type cells or with another strain (BTU2 null) expressing the *neo* gene inserted at a different chromosomal location (Fig. 2D). The more rapid loss in viability of G8 null cells was not due to a failed heat shock response in starvation medium (Fig. 2E), but rather was correlated with a loss in overall protein synthesis capacity at 39°C (Fig. 2F).

An apparently universal outcome of the stress response induced at sublethal temperatures is thermotolerance, an increase in the viability of cells subsequently exposed to normally lethal temperatures (*1*). In *T. thermophila*, two forms of thermotolerance have been demonstrated. In the first, a prior sublethal hsp-inducing treatment increases the viability of cells subsequently exposed to 45° to 49°C, temperatures at which protein synthesis is always completely inactivated (*15*, *16*). To establish thermotolerance of this

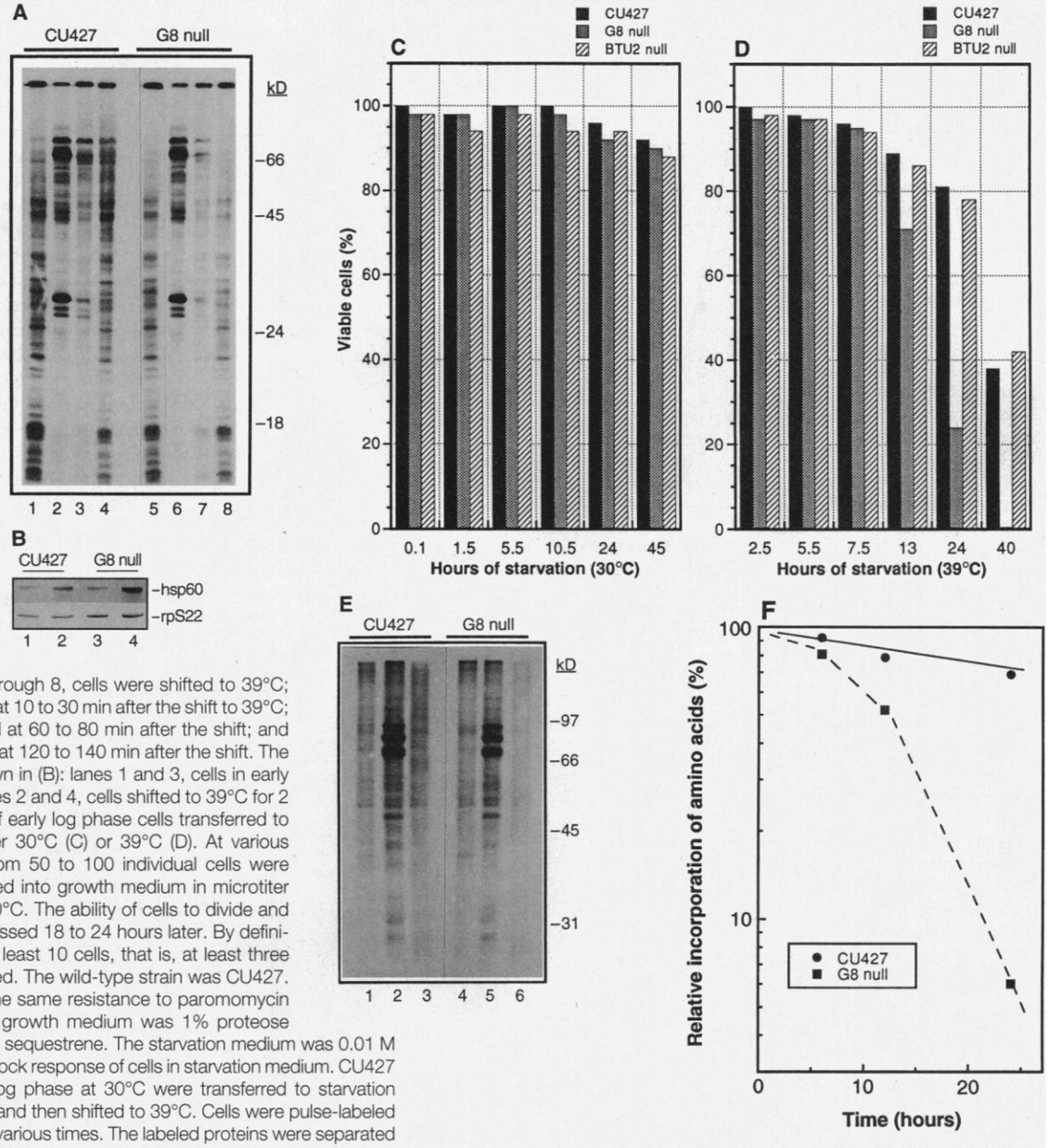

Fig. 2. Viability and metabolic properties of G8 null and control cells exposed to starvation and heat shock. (**A** and **B**) Heat shock response of CU427 and G8 null cells. Cells in early log phase growth at 30°C were shifted to 39°C and pulse-labeled with [^{3}H]lysine, as in (*7*), before and at times after the temperature shift. Solubilized proteins were separated by SDS–polyacrylamide gel electrophoresis (PAGE) on 12% gels and prepared for fluorography as in (*7*) or transferred to filters and immunodecorated with either antibody to hsp60 (anti-hsp60) or, as an internal control, antibody to ribosomal protein S22 (rpS22) as in (*7*). The fluorogram is shown in (A), lanes 1 and 5, cells labeled for 20 min at 30°C; lanes 2 through 4 and 6 through 8, cells were shifted to 39°C; lanes 2 and 6, cells labeled at 10 to 30 min after the shift to 39°C; lanes 3 and 7, cells labeled at 60 to 80 min after the shift; and lanes 4 and 8, cells labeled at 120 to 140 min after the shift. The protein immunoblot is shown in (B): lanes 1 and 3, cells in early log phase at 30°C; and lanes 2 and 4, cells shifted to 39°C for 2 hours. (**C** and **D**) Viability of early log phase cells transferred to starvation medium at either 30°C (C) or 39°C (D). At various times after the transfer, from 50 to 100 individual cells were isolated by hand, transferred into growth medium in microtiter plates, and incubated at 30°C. The ability of cells to divide and produce colonies was assessed 18 to 24 hours later. By definition, a colony contained at least 10 cells, that is, at least three cellular fissions had occurred. The wild-type strain was CU427. The BTU2 null strain had the same resistance to paromomycin as the G8 null strain. The growth medium was 1% proteose peptone containing 0.003% sequestrene. The starvation medium was 0.01 M tris-HCl (pH 7.4). (**E**) Heat shock response of cells in starvation medium. CU427 and G8 null cells in early log phase at 30°C were transferred to starvation medium at 30°C for 1 hour and then shifted to 39°C. Cells were pulse-labeled for 20 min with [^{3}H]lysine at various times. The labeled proteins were separated by SDS-PAGE (10% gel) and visualized by fluorography as in (A). Lanes 1 and 4, cells labeled at 30°C; lanes 2 and 5, cells labeled 20 to 40 min after the shift to 39°C; and lanes 3 and 6, cells labeled 280 to 300 min after the shift to 39°C. (**F**) Amino acid incorporation in CU427 and G8 null cells in starvation medium at 39°C. Cells were labeled as in (E) at times after a shift to 39°C. The trichloroacetic acid–insoluble counts per minute incorporated at the various times was determined and plotted as a percentage of the counts per minute incorporated during a labeling of cells at 20 to 40 min after a shift to 39°C.

kind, cells must pre-accumulate hsps. Operationally, this form of thermotolerance is the one most frequently measured in other systems (*1*). G8 null cells tested for this form of thermotolerance were indistinguishable from wild-type cells (*17*), again demonstrating that, with respect to hsp production, their heat shock response is normal. In the second form of thermotolerance, an appropriate pretreatment endows cells with an increased viability at 42° to 44°C. The difference is that in treated cells, but not in untreated cells, protein synthesis is maintained at the high temperature and hsps are produced. In this latter case, unlike the first form of thermotolerance, an appropriate pretreatment does not necessarily elicit the prior accumulation of hsps (*16, 18*). The crucial characteristic of this second form of thermotolerance is that hsp synthesis occurs at the elevated temperature. The conditions that elicit this form of thermotolerance all appear to alter ribosome structure (*7, 14, 18, 19*).

Because protein synthesis was more thermolabile in G8 null cells exposed to a normally nonlethal heat shock treatment, we investigated whether the induction of the second form of thermotolerance was in any way compromised in G8 null cells. Cells in growth medium at 30°C were shifted to 39°C for 1 hour and then transferred to 43°C, or they were shifted directly from 30°C to 43°C, and their viabilities were determined as a function of time at the elevated temperature. In comparison with control cells, the G8 null cells showed a reduced capacity to survive at 43°C (Fig. 3A). When we performed the same thermotolerance assay on cells in starvation medium, an even greater reduction in survival of the G8 null cells was evident (Fig. 3B).

We next determined whether this loss of viability was due to the inability of G8 null cells to thermoprotect their translational machinery. When we measured the capacity of cells to maintain protein synthesis at 43°C, control cells given a 39°C pretreatment showed only a moderate loss in their ability to synthesize proteins at 43°C (Fig. 4A). In contrast, whereas the pre–heat shocked G8 null cells initially synthesized hsps at 43°C in a manner similar to the control cells (Fig. 4B), they were unable to maintain protein synthesis (Fig. 4A), although, when compared with the nonpretreated controls, a partial degree of protein synthesis stabilization was apparent.

These results indicate that G8 RNA plays a role in maintaining a functional translational apparatus at high temperature. However, other factors must contribute to this stabilization, as G8 null cells can develop some thermotolerance, the extent of which is affected by their physiological state (Fig. 3, A and B). Evidence for such factors comes from a mutant strain of *T. thermophila*, MC3 (*18*), in which the first but not the second type of thermotolerance can be induced. The MC3 strain exhibits a phenotype remarkably similar to the G8 null strain, except that G8 RNA accumulation and ribosome association are normal. Finally, our results disprove our original hypothesis that G8 RNA is required for selective mRNA translation during stress conditions.

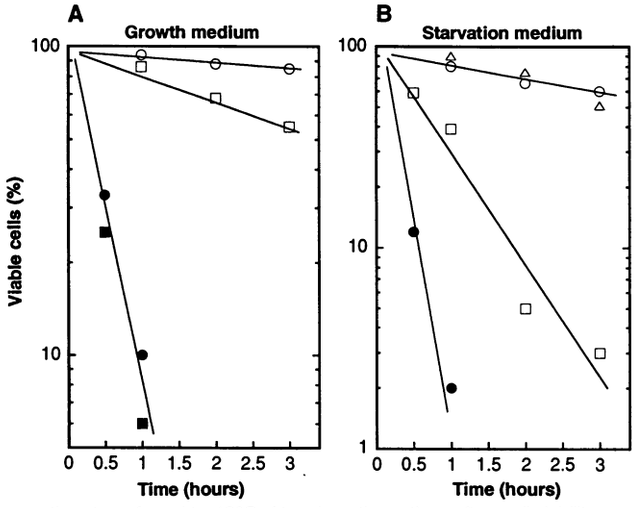

Fig. 3. Viability of G8 null and wild-type cells at 43°C. (**A**) A culture of cells in early log growth at 30°C was divided in half: one half was transferred to 39°C, the other remained at 30°C. After 1 hour, both cultures were transferred to 43°C. Cell viability, the percentage of cells giving colonies at 30°C, was measured as in Fig. 2. (**B**) Cells in log growth at 30°C were collected, washed, transferred into starvation medium, and incubated at 30°C for 1 hour. The culture was then divided in half: one half remained at 30°C, the other half was transferred to 39°C for 1 hour. Both cultures were then transferred to 43°C. At various times thereafter, cell viability was measured as above. The growth and starvation media were as described in Fig. 2. (○, ●) CU427 cells, (□, ■) G8 null cells, and (△) BTU2 null cells. The closed symbols are for the shift from 30°C to 43°C and the open symbols are for the shifts from 30°C to 39°C to 43°C.

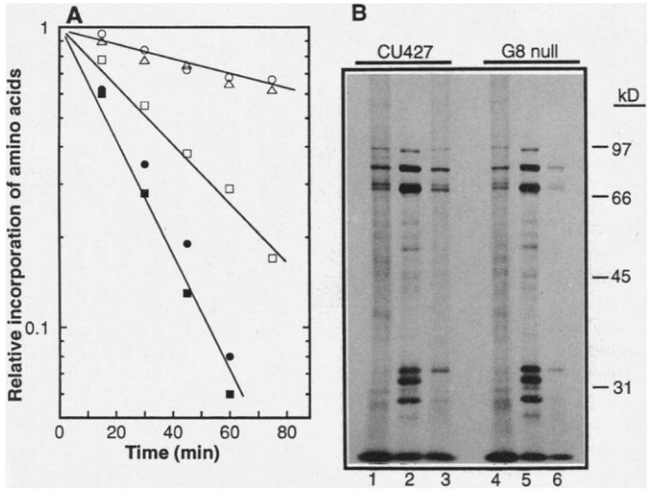

Fig. 4. Protein synthesis in G8 null and control cells at 43°C. (**A**) Cells in early log phase at 30°C were transferred to starvation medium and treated as in Fig. 3B. At various times after the shift from 39°C to 43°C, the relative rate of protein synthesis was determined for each culture and expressed as a fraction of the rate displayed by cells labeled at zero time (*20*). The symbols are the same as in Fig. 3. (**B**) Fluorographic patterns of proteins synthesized in CU427 and G8 null cells before and after the shift to 43°C. Fluorography of labeled proteins was as above. Lanes 1 and 4, cells labeled for 20 min after being at 39°C for 1 hour; lanes 2 and 5, cells labeled at 10 to 30 min after the shift to 43°C; lanes 3 and 6, cells labeled at 40 to 60 min after the shift to 43°C.

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13. A chimeric gene composed of the 5′ regulatory sequences of the *T. thermophila* histone H4-1 gene (5′ H41), the coding region of the *neo* gene from the Tn5 transposon, and the 3′ flanking sequences of the *T.*

thermophila β-tubulin-2 gene containing an ATG translation terminator (3′ BTU2) was constructed as in (*10*). A Bluescript KS(+) plasmid (pBS-G8) containing a chromosomal copy of the G8 gene in the middle of a 1.3-kb Sal I–Bam HI fragment was linearized within the G8 coding sequence by digestion with Sty I. The staggered ends were filled in with Klenow, and a Sma I–Eco RV fragment containing the H41-neo-BTU2 chimeric gene was ligated into the linearized pBS-G8 plasmid. The resulting plasmid, pG8-neo, was digested with Sal I and Bam HI liberating a 2.7-kb fragment containing the disrupted G8 gene and its normal flanking sequences. This DNA was used to transform, by electroporation, the *T. thermophila* strain CU427 (*11*). Transformants were initially selected with paromomycin (120 μg/ml) in the growth medium. Further selection in higher concentrations of paromomycin (up to 750 μg/ml) produced cell lines that remained resistant to a concentration of paromomycin of 750 μg/ml even when grown for 30 to 40 generations in the absence of drug. These lines were designated G8 null cells. BTU2 null cells are a strain of *T. thermophila* developed in the Gorovsky lab in which the β-tubulin-2 gene contains a *neo* insertion. They are otherwise isogenic with CU427 and G8 null cells.

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17. E. M. Hallberg and R. L. Hallberg, unpublished data. Cultures of early log phase G8 null and CU427 cells at 30°C were either maintained at that temperature or were shifted to 39°C for 1 hour. All cultures were then transferred to 46°C, and at 3-min intervals thereafter (for a total of 12 min), samples of cells were removed from each culture; individual cells from each sample were then transferred to growth medium in microtiter plates and incubated at 30°C. Twenty-four hours later, the fraction of viable cells was determined. Both G8 null and CU427 cells given a 1-hour prior heat shock exhibited a degree of thermotolerance similar to that shown for a number of other strains (*15, 18*).
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20. From individual cultures of cells in starvation medium at 43°C, 0.2-ml samples were removed at various times and added to 0.05 ml of prewarmed starvation medium containing [^{3}H]lysine (100 μCi/ml). Incubation was continued at 43°C for 10 min, after which triplicate 75-μl samples were removed and trichloroacetic acid (TCA) added to 10%. The insoluble material was collected on filters, washed, dried, and counted. This TCA-insoluble incorporation was used as an estimate of the rate of protein synthesis. The values plotted are calculated from the means of the counts per minute in the three samples collected.

21. We thank E. M. Hallberg for her expert technical assistance in carrying out the thermotolerance experiments. This work was supported by NIH grants GM46302 (to R.L.H.) and GM26973 (to M.A.G.).

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Structure of a Complex of Two Plasma Proteins: Transthyretin and Retinol-Binding Protein

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The three-dimensional structure of the complex formed by two plasma proteins, transthyretin and retinol-binding protein, was determined from x-ray diffraction data to a nominal resolution of 3.1 angstroms. One tetramer of transthyretin was bound to two molecules of retinol-binding protein. The two retinol-binding protein molecules established molecular interactions with the same transthyretin dimer, and each also made contacts with one of the other two monomers. Thus, the other two potential binding sites in a transthyretin tetramer were blocked. The amino acid residues of the retinol-binding protein that were involved in the contacts were close to the retinol-binding site.

Transthyretin (TTR, formerly called prealbumin), one of the transporters of the hormone thyroxine, and retinol-binding protein (RBP), the specific carrier of retinol (vitamin A), form a complex under physiological conditions that prevents the glomerular filtration of the low molecular size RBP (21,000 daltons) in the kidneys (*1*). The complex can form in vitro between RBP and TTR from different species, including those species that are distant in evolution (*2*). We prepared crystals from complexes containing human TTR and chicken RBP (*3*). The dissociation constant of this complex (1.0×10^{-7} M) is similar to those of the complexes of human RBP with human TTR (1.1×10^{-7} to 1.5×10^{-7} M) and of chicken RBP with chicken TTR (1.1×10^{-7} to 1.6×10^{-7} M) (*4*).

The crystallographic data of this complex are presented in Table 1. The structure of the complex was solved with the molecular replacement method (*5*) with one of the human TTR coordinate sets (*6*) and coordinates of bovine holo-RBP (*7*). The final model statistics (*8*) are an *R* factor of 20.1 (*R* factor $= \Sigma \mid F_o - F_c \mid / \Sigma \mid F_o \mid$ where F_o is the observed and F_c the calculated structure factor) with root-mean-square deviations in the bonds of 0.018 Å, in the angles of 3.7°, and in the dihedrals of 26.7°.

The three-dimensional structure of human (*9, 10*) and bovine (*7*) holo- and apo-RBP, of wild-type (*11*) and several mutant human TTRs (*12, 13*), and of complexes of wild-type TTR with several pharmacologically important compounds (*6, 14*) are all known. TTR is a tetramer of four identical subunits, each 127 amino acids long (Fig. 1) (*11*). The monomers are organized into two extensive β sheets, each composed of four strands that are all antiparallel, with one exception (*11*). Two monomers form a stable dimer by the extension of their two β sheets through hydrogen bonding that involves the four strands (two from each monomer) at the edges of the two subunits. The two dimers of the tetramer are separated by a channel and in contact through symmetry related loops. The channel, approximately 10 Å in diameter, is the ligand-binding site

Fig. 1. Model of the structure of the hexameric complex $(RBP)_2$-TTR as determined by x-ray analysis of the orthorhombic crystals. The RBP molecules are shown in red, one of the TTR dimers is in green and yellow, and the other is in blue and turquoise. The retinol molecules are represented as space-filling models with white carbon atoms and a purple oxygen. The view is looking down the *z* axis of the TTR tetramer as defined by Blake and co-workers (*11*). The *x* and *y* axes are in the plane of the figure, horizontally and vertically, respectively. They intercept in the center of the channel, which is the hormone-binding site, that runs through the TTR tetramer and is empty in the figure.

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