Levels of Major Proteins of *Escherichia coli* During Growth at Different Temperatures

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The adaptation of *Escherichia coli* B/r to temperature was studied by measuring the levels of 133 proteins (comprising 70% of the cell's protein mass) during balanced growth in rich medium at seven temperatures from 13.5 to 46°C. The growth rate of this strain in either rich or minimal medium varies as a simple function of temperature with an Arrhenius constant of approximately 13,500 cal (ca. 56,500 J) per mol from 23 to 37°C, the so-called normal range; above and below this range the growth rate decreases sharply. Analysis of the detailed results indicates that (i) metabolic coordination within the normal (Arrhenius) range is largely achieved by modulation of enzyme activity rather than amount; (ii) the restricted growth that occurs outside this range is accompanied by marked changes in the levels of most of these proteins; (iii) a few proteins are thermometer-like in varying simply with temperature over the whole temperature range irrespective of the influence of temperature on cell growth; and (iv) the temperature response of half of the proteins can be predicted from current information on their metabolic role or from their variation in level in different media at 37°C.

In general, individual bacteria can grow over a range of approximately 40 (Celsius) degrees (23). For *Escherichia coli*, a typical mesophile, balanced growth can be sustained from 10 to almost 49°C (depending at the upper extreme on the nutrients present in the medium). In the middle of this temperature range—from approximately 20 to 37°C—the rate of growth of *E. coli* varies with temperature as though cellular growth, no matter what the medium, were a simple chemical process with a temperature characteristic (μ) of 12,000 to 14,000 cal (ca. 50,230 to 58,600 J) per mol (8). Increasing the temperature above 40°C, or decreasing it below 20°C, leads to progressively slower growth until finally growth ceases altogether at 9 or 49°C.

It is not known how *E. coli* manages its metabolic affairs so as to maximize growth rate between 20 and 37°C, nor is it understood what prevents this optimization outside this range. A prevalent and reasonable view is that one or more reactions become rate limiting above 37°C (16) and below 20°C (2, 3, 8, 13) as a result of the inability of the cell to compensate for thermally (hot or cold) induced changes in conformation of proteins. The "normal" or "Arrhenius" range in this view is therefore the range over which the cell is successful in adjusting reaction rates that get out of line: adjustment that might entail changing the amount of the proteins involved, their activity, or both.

Experimental exploration of this view has not been extensive, largely because of the previous inadequacy of methods to measure the levels of many enzymes simultaneously. Work has mostly been directed at the sub- and supranormal temperatures to learn what proteins might be limiting growth. An initiation factor in protein synthesis has been implicated as the vulnerable element both at high (16) and low (2) temperature. Other work has shown that growth at high temperature in minimal medium can be restricted by inactivation of a (methionine) biosynthetic enzyme (20, 21). In some sense the isolation of mutants with temperature-sensitive enzymes has provided a ready source of possible analogs of the wild-type cell at high and at low temperature (e.g., ref. 7). No systematic study has been made of the levels of individual proteins as a function of temperature within or beyond the normal range, but Schaechter et al. (22) showed that cell volume, mass, RNA, DNA, and the number of nuclei per cell in *Salmonella typhimurium* were nearly constant for a given medium at 37 and 25°C.

With the advent of methods for resolving total cell protein (14, 15) and accurately measuring their levels (e.g., ref. 17), comprehensive studies are now possible. We recently reported unexpected changes in transient rates of synthesis of individual proteins after quite modest shifts in growth temperature (9). Here we present the first picture of how the levels of the major proteins of *E. coli* vary during steady-state growth

at different temperatures within and beyond the normal, Arrhenius range.

MATERIALS AND METHODS

Bacterial strain. The *E. coli* B/r derivative NC3 (11) was used in all experiments.

Media. All media used were based on the defined MOPS medium (11) and were made by supplementing the MOPS medium with 0.4% glucose (wt/vol), amino acids (minus leucine; 0.12 mM valine and 0.08 mM isoleucine), five vitamins, and four bases in concentrations given previously (26). At 13.5, 15, 42, and 46°C twice the normal concentration of MOPS was necessary to obtain steady-state growth at the desired cell densities.

Bacterial growth. Cells were grown aerobically on a rotary shaker at seven temperatures. The temperature was controlled to within ±0.1°C with a thermistor probe. All cultures were started at an optical density at 420 nm of 0.01 and grown to an optical density of 1.0, approximately 10^8 cells per ml. In all cases growth was monitored with flasks growing in parallel to the flask containing radioactive isotope.

Radioactive labeling. Steady-state cultures of strain NC3 were grown in rich medium containing [^{14}C]leucine (356 mCi/mmol; 40 μCi/ml) at each of seven chosen temperatures (13.5, 15, 23, 30, 37, 42, and 46°C). A reference culture was prepared at 37°C in the same medium but with [^{3}H]leucine (502 mCi/mmol; 63 μCi/ml). Cells were harvested, and portions of cells from the reference culture were mixed with cells of each of the seven experimental cultures.

Determination of steady-state levels. Extracts were prepared in the manner described by Blumenthal et al. (1). Portions (20 μl) of extracts were processed by the O'Farrell equilibrium and nonequilibrium gel systems (14, 15). The amounts of ^{14}C and ^{3}H isotopes in the individual protein spots and in the unresolved extracts were measured, after sample oxidation, in the manner described by Lemaux et al. (9). The ^{14}C:^{3}H value for each protein, divided by the ^{14}C:^{3}H ratio of the unresolved mixture, is the level of that protein in the experimental culture relative to the reference culture.

RESULTS

The growth rate of *E. coli* NC3 (or its close derivative strain NC81) was measured in a series of steady-state cultures at different temperatures in rich and in minimal media. The log of the specific growth rate constant (k) is plotted as a function of the inverse of absolute temperature in Fig. 1. This function appears linear between 21 and 37°C, and has approximately the same μ value (13,000 to 14,000 cal; ca. 54,400 to 58,600 J) in rich and in minimal medium. On the basis of this information, three temperatures within the linear range were chosen for study: 23, 30, and 37°C. Outside this range four temperatures were chosen to provide cells with various levels of restricted growth (defined as percentage of the value predicted at each tempera-
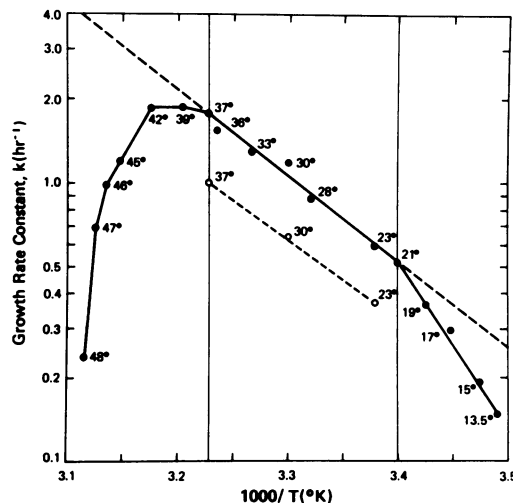

FIG. 1. *Growth rate of E. coli B/r as a function of temperature. Cultures were grown to steady state at each temperature, and the rate of growth was measured. The logarithm of the growth rate constant, k (h^{-1}), is plotted on the ordinate against the inverse of the absolute temperature (°K) on the abscissa. Individual data points are marked with the corresponding degrees Celsius. (●) Strain NC3 in glucose-rich medium; (○) strain NC81 (identical to strain NC3 except for lacI lacP37 lacP5 thi) in glucose minimal medium.*

ture by extrapolating the linear Arrhenius relationship): 13.5°C, 56% normal rate; 15°C, 63% normal rate; 42°C, 72% normal rate; and 46°C, 28% normal rate.

The steady-state levels of individual proteins resolved by the O'Farrell technique were measured at each of the seven temperatures relative to their levels in reference cells growing at 37°C. The results are presented in Table 1, which also contains, where available, the identification of the individual proteins, their metabolic class, and their chemical abundance in the cell. We have prepared several additional displays of these data to aid in analysis.

In Fig. 2 we have grouped the proteins according to the magnitude of variation in their level within the normal range of temperature, 23 to 37°C. This histogram reveals that the amounts of most of the 111 measured proteins change very little throughout the normal Arrhenius temperature range: only 2 change more than 2.5-fold, and they change only 4-fold, and 83 of the 111 change less than 1.6-fold. No transcription or translation protein changed more than 1.4-fold, and most changed 1.2-fold or less.

A similar histogram has been prepared for the 133 proteins for which we have data over the entire range of 13.5 to 46°C. Figure 3 displays

TABLE 1. *Steady-state levels of individual proteins*

Protein Number[a]	Protein Identification[b]	Metabolic Regulation Group[c]	Weight fraction of total protein in glucose rich medium at 37°C[d] α' (μg/mg)	Level at each temperature relative to level at 37°C					
				13.5°C	15°C	23°C[e]	30°C	42°C	46°C
A13.0	L7	Ic	3.05	2.21	-	- (0.96)	1.01	0.82	0.50
A165		-	-	0.91	0.24	0.79	0.89	0.77	25.03
B13.0	L12	Ic	9.96	0.25	-	- (1.10)	0.74	0.68	0.37
B18.4		Ia4	1.68*	3.40	1.92	1.60	1.30	1.02	1.49
B18.7		Ib	1.44*	1.28	0.66	0.63	1.11	1.17	1.35
B20.9		IIb1	7.17*	0.95	0.68	0.69 (0.95)	0.87	1.38	2.16
B35.1		Ib	0.85*	0.63	0.62	0.56 (0.54)	0.86	1.26	0.93
B40.7	RNP, α	Ic	5.27	0.89	0.87	0.99 (0.99)	1.19	1.03	1.05
B46.7	ATPase, α	Ia4	5.30	1.15	1.26	1.29	1.18	0.96	1.69
B50.3		Ic	9.66	1.19	1.08	1.31 (1.17)	1.28	0.84	0.62
B56.5	A, groE	Ic	16.47	0.94	0.85	0.61 (0.68)	0.76	1.65	7.22
B61		Ic	3.01*	1.16	1.05	1.14	1.07	0.94	0.52
B65	S1	Ic	26.27	0.73	0.78	0.97 (1.16)	1.00	0.97	0.46
B66		Ic	14.09	0.66	0.69	0.63 (0.59)	0.78	1.42	3.06
C15.3		Ic	0.68	0.63	-	-	0.55	1.30	5.65
C22.7		Ic	2.04*	0.58	0.62	0.89	0.93	1.02	1.11
C30.7	EF-Ts	Ic	2.12	0.80	0.55	0.88 (0.82)	1.00	1.00	0.55
C31.6	EF-Ts	Ic	5.65	0.84	0.79	0.79 (0.82)	1.02	1.02	0.60
C34.3		Ib	2.39	0.42	1.01	1.02	1.00	1.19	0.81
C40.3[f]		Ib	5.17	1.48	0.73	0.68 (1.07)	0.85	1.15	1.90
C44.2		IIa1	3.12*	0.84	0.84	1.08	1.13	0.79	0.65
C44.6		IIa1	4.01	0.44	0.49	0.73	0.88	1.24	0.66
C56		-	1.68*	2.06	1.25	1.18	1.10	1.02	0.29
C58.5		-	2.61*	1.59	1.32	1.11	1.02	1.12	1.10
C60.7		Ic	4.18*	0.57	0.62	0.70	0.82	0.83	0.55
C62.5		Ic	4.45*	0.49	0.59	0.47 (0.46)	0.51	1.94	2.73
C62.7		Ic	2.18*	0.79	0.61	0.59	0.87	1.09	0.63
C70		Ic	3.27*	0.73	0.59	0.64	0.85	1.11	0.69
C78		Ic	4.29*	3.22	3.21	2.01	1.32	0.93	0.56
D31.5		IIb1	1.36	0.63	0.63	0.75	0.97	1.27	1.98
D32.5		IIa1	1.24	0.53	0.57	0.68 (0.89)	0.83	1.16	1.06
D40.7		Ia3	1.05	5.60	4.03	1.53 (1.19)	1.05	1.34	4.35
D44.5		-	2.71*	2.30	1.45	1.32	1.27	1.46	2.06
D46		-	2.55*	0.89	0.75	0.65	1.02	1.21	3.06
D47		-	2.82*	1.49	1.03	0.75	0.93	1.18	1.02
D47.5		-	2.38*	1.63	0.97	0.99	1.02	1.08	1.50
D49.2		-	1.31*	1.12	1.02	0.85	0.97	1.08	0.93
D58.5	LysS	Ic	1.48	0.88	0.85	0.99 (1.04)	1.04	0.90	0.49
D74		Ic	4.99	2.16	2.16	1.82	1.45	0.57	0.24
D84	EF-G	Ic	28.22	1.02	1.08	1.10 (1.09)	1.12	0.94	0.49
D87.5		Ib	2.45*	0.72	1.03	1.08	1.09	0.79	0.65
D94	PheS, β	Ic	2.26	0.75	0.89	0.89 (0.97)	1.08	1.08	0.58
D99		Ia3	1.05	1.88	2.05	1.79 (1.35)	1.41	0.81	1.36
D100	LeuS	Ic	2.05*	0.73	1.03	1.29 (0.99)	0.94	0.90	0.60
D157	RNP, β	Ic	7.43	1.04	1.01	1.20 (1.24)	1.09	0.90	0.60
E22.8		IIa1	0.64*	0.58	0.51	0.74	0.88	0.82	0.50
E23		Ib	1.08*	0.55	0.67	0.82	0.87	0.81	0.51
E24.8		Ib	1.25*	1.08	0.87	0.95	0.94	1.24	1.80
E38.5		-	0.63	1.41	0.76	0.97	1.17	0.93	1.06
E42	EF-Tu	Ic	84.92	0.89	0.77	0.85 (0.84)	1.02	1.05	0.93
E48.7		-	2.69*	1.12	1.14	1.42	1.32	1.49	2.63
E58	ArgS	Ic	1.37	0.73	0.84	0.93 (0.96)	0.91	0.96	0.59
E77.5	GlyS	Ic	2.74	0.49	0.59	0.71 (0.87)	0.77	1.19	0.82
E79		IIb2	4.69	0.36	0.49	0.52	0.69	1.16	0.55
E106	ValS	Ic	2.11	0.78	0.79	1.17 (1.09)	1.05	1.22	0.85
E133		IIa2	0.86	0.64	0.98	1.68	1.52	0.85	2.47
E140		-	1.11	0.56	0.71	0.92	0.91	1.09	0.66
F14.7		Ia4	0.85*	1.28	-	-	1.00	0.78	0.58
F24.5		Ib	16.20	2.47	1.29	0.99 (1.24)	1.11	1.37	2.42
F24.6		-	1.20*	1.43	1.15	1.01	1.05	1.15	2.40
F26		-	1.41*	1.49	1.00	0.98	1.11	1.07	1.01
F30.2		Ic	4.02	0.73	0.59	0.65	0.82	0.97	0.46
F32.3		Ia3	0.48	2.03	1.61	0.60	0.82	2.04	9.03
F32.5		IIa2	2.06	1.27	1.15	1.41 (1.17)	1.35	1.28	2.75
F38		IIa2	2.94	1.85	1.51	1.20	1.30	1.08	1.16
F39		Ib	2.74*	2.81	1.52	1.22	1.23	1.16	1.46
F42.2		-	6.40*	0.76	0.74	0.82	0.92	1.01	0.91

TABLE 1—*Continued*

Protein Number[a]	Protein Identification[b]	Metabolic Regulation Group[c]	Weight fraction of total protein in glucose rich medium at 37°C[d] a' (µg/mg)	13.5°C	15°C	23°C[e]		30°C	42°C	46°C
F43.8		Ic	10.54*	1.44	1.51	1.43		1.04	1.11	1.23
F43.9		Ib	3.53	1.74	1.33	1.28		1.43	0.79	0.58
F48.1	GluS, β	Ic	1.51	0.95	0.70	0.81	(0.87)	0.75	1.15	0.78
F50.3		Ia3	0.24	2.64	1.95	1.15		0.87	1.22	2.84
F56		Ia2	2.00*	0.68	1.14	0.98		0.95	1.27	1.86
F56.2		Ic	5.03	0.63	0.65	0.86		0.94	1.03	0.63
F56.5		-	1.69	0.81	0.61	0.98		1.23	1.39	1.87
F58.5	AspS	Ic	1.64*	1.48	1.15	1.25	(1.13)	1.22	0.85	0.46
F60.3		Ia3	0.23	2.16	1.70	1.00		0.93	1.45	2.76
F63.4		Ic	0.56	0.66	0.99	0.78		1.23	0.90	0.97
F63.5		-	1.24	0.57	1.04	0.80		0.77	0.88	0.97
F63.8		Ia4	1.78	1.29	0.74	0.83		0.89	1.18	0.73
F64.5		IIa1	2.91	0.82	0.94	0.89		0.97	0.89	0.70
F82.5		Ia3	0.19	5.35	4.06	2.15	(2.20)	1.13	1.17	1.59
F84		IIa2	0.79	2.20	1.58	1.28		1.25	1.01	1.84
F84.1		Ia4	0.73	0.28	0.35	0.46	(2.41)	0.51	0.94	4.66
F88		IIa3	1.13	2.79	5.71	4.18	(1.34)	1.02	0.88	0.57
F99		Ic	24.12	0.69	0.58	0.62		0.71	0.98	0.47
F107	IleS	Ic	2.79	1.08	1.23	1.20	(1.26)	1.08	0.78	0.83
F178		Ib	0.63	1.90	2.42	1.58		1.20	-	0.53
G25.3		Ib	2.01	1.14	0.80	0.78		1.04	1.27	1.54
G27.2		Ia1	0.45*	3.19	3.10	2.30	(2.31)	1.52	0.91	0.30
G29.6		-	0.50*	23.90	1.77	1.20		0.94	1.44	0.84
G32.8		-	0.75	0.65	1.41	0.96	(0.58)	0.66	0.38	0.56
G36	PheS, α	Ic	1.12	1.16	0.77	0.78	(0.75)	0.82	1.07	0.69
G41		-	1.55*	1.79	0.49	0.77		1.08	0.95	2.92
G41.2		Ib	2.07	5.95	1.40	1.16		1.02	1.01	0.89
G41.3		Ic	5.49	0.28	0.39	0.47		0.70	0.93	0.25
G41.4		IIa2	0.41	2.30	0.93	1.53		1.34	0.85	2.87
G43.2		IIa2	1.01	0.96	1.88	1.75		1.43	1.52	1.08
G43.8		IIa2	1.99	1.42	0.99	1.28		1.19	1.12	2.15
G43.9		Ia4	3.54*	0.55	0.42	0.49		0.73	1.00	0.91
G44		Ib	2.69*	0.57	0.54	0.72		0.86	1.13	1.16
G50.5		IIb1	6.49	0.94	0.72	0.75		1.09	1.18	1.20
G51	ATPase, β	Ia4	6.00	0.99	1.19	1.27		1.21	0.99	1.73
G54.7		Ic	3.62	1.37	0.89	0.86		0.95	1.02	0.91
G57		Ic	0.54	0.59	0.86	1.12		1.08	0.97	0.72
G61	GlnS	Ic	1.49	0.93	0.82	0.83	(0.95)	0.85	0.94	0.83
G62.8		Ia4	.38	1.68	0.82	0.72		0.78	0.64	1.75
G74		Ia2	2.34	1.66	3.21	3.47	(2.99)	0.95	0.77	1.54
G76		Ia3	-	1.83	1.77	1.17		1.24	1.07	1.24
G78		Ic	0.88	0.69	0.74	1.26		1.13	1.04	0.97
G93		Ia4	1.16	0.78	0.80	0.91		1.00	1.13	1.17
G97		Ia3	0.60	2.42	2.68	1.46	(0.89)	0.90	1.12	1.41
G117		Ic	-	1.79	1.65	1.34	(1.14)	1.09	1.04	1.03
G127		Ib	-	1.08	1.01	1.04		0.89	0.99	0.99
H52.7		Ic	4.07	0.31	0.41	0.62		0.98	1.32	0.36
H54.6		Ia2	0.53	1.08	2.10	1.89	(0.38)	1.38	1.08	1.52
I14.2	Ribosomal Pro.	Ic	-	0.78	-	-		1.08	0.99	0.49
I14.4	Ribosomal Pro.	Ic	-	0.75	-	-		1.05	0.98	0.55
I14.7	Ribosomal Pro.	Ic	-	0.63	-	-		1.03	0.93	0.43
I16.4	Ribosomal Pro.	Ic	-	0.69	-	-		0.96	1.07	0.54
I17.2	Ribosomal Pro.	Ic	-	0.74	-	-		0.99	1.00	0.47
I19.5		-	-	2.06	-	-		1.33	1.28	1.02
I21.1		-	-	0.58	-	-		0.82	0.97	0.50
I21.3		-	3.91*	1.41	-	-		1.06	0.69	0.64
I21.4		-	-	0.98	-	-		0.90	0.75	1.30
I23.0	Ribosomal Pro.	Ic	-	0.66	-	-		0.99	1.02	0.49
I26.0	Ribosomal Pro.	Ic	-	0.65	-	-		0.98	0.97	0.45
I26.3	Ribosomal Pro.	Ic	8.63*	0.76	-	-		1.01	0.96	0.48
I33.5		-	8.58*	1.50	-	-		1.00	1.04	1.28
I35.2		Ib	-	1.15	-	-		0.95	1.17	1.25
I47.2		Ic	5.60*	0.93	-	-		0.98	1.08	0.85
I49.6		Ic	1.24*	0.70	-	-		1.12	0.91	0.41
I58.4		-	2.34*	0.13	-	-		0.90	0.05	0.05
I63.5		Ic	1.71*	5.82	-	-		1.64	0.77	0.42

[a] Proteins are numbered as described in Pedersen et al. (17). Letter prefixes refer to the position of the protein in the axis parallel to the isoelectric focusing dimension (A–I indicates progression from acid to base);

TABLE 1—*Continued*

numbers refer to the apparent molecular weight of the protein as determined by the distance moved in the sodium dodecyl sulfate electrophoresis dimension (56.5 indicates molecular weight of 56,500).

[b] Abbreviations used: S—aminoacyl-tRNA synthetase; L7, L12, S1—identified ribosomal proteins; Ribosomal Pro.—proteins known to be ribosomal proteins, but not identified further; RNP, α—RNA polymerase, α subunit; RNP, β—RNA polymerase, β subunit; EF-Ts, EF-Tu, EF-G—protein synthesis elongation factors Ts, Tu, and G; A, *groE*—the A protein of Subramanian et al. (25), also identified as the product of the *groE* gene by Drahos and Hendrix (personal communication).

[c] The proteins have been grouped according to the way in which their relative levels change in cells grown in different media. The numerical values are published in Pedersen et al. (17), and the behavior of each class is shown in Fig. 6.

[d] Most of these values were measured by determining the total radioactivity in individual spots on a gel made from cells grown on uniformly labeled [^{14}C]glucose, and are taken from Table 1 of Pedersen et al. (17). The values with an asterisk were measured in the same manner, but with cells grown on [^{14}C]leucine; these values are influenced by any differences in the leucine content of the individual proteins relative to the average for *E. coli* protein, and therefore should be regarded as approximate.

[e] The values in parentheses were measured in a separate experiment (strain NC81) in which both the experimental culture (23°C) and the reference culture (37°C) were grown in glucose minimal medium.

[f] Protein spot C40.3 has recently been recognized to contain, in most gels, two individual proteins. The data shown reflect the behavior of the dominant protein of this pair, but must be regarded as approximate.
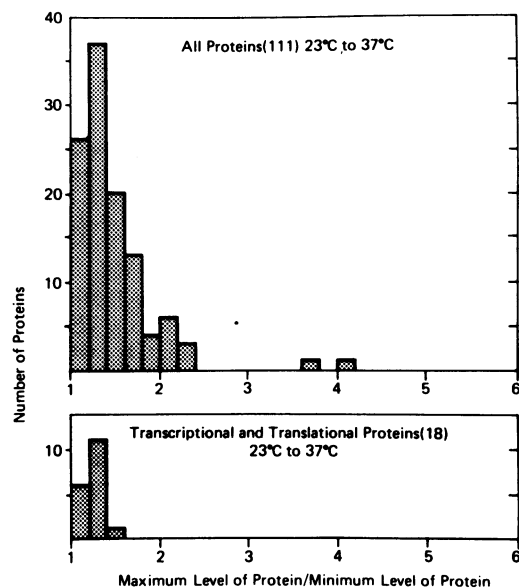

FIG. 2. *Distribution of E. coli proteins with respect to magnitude of variation in level within the normal temperature range, 23 to 37°C. For each protein its maximum level between 23 and 37°C was divided by its minimum level in this range. Proteins were then grouped according to the magnitude of their variation: 1.0–1.2, 1.2–1.4, 1.4–1.6. The upper panel shows all 111 proteins for which data are available; the lower panel shows 18 transcriptional and translational proteins. All data were taken from Table 1.*

what is evident to the unaided eye from the autoradiograms of the O'Farrell gels; the relative abundance of individual proteins at 46°C is easily distinguished from that at 37°C. Of 133 proteins, 83 vary more than 2-fold, 18 vary more than 5-fold, and 9 vary more than 10-fold. The two proteins with the greatest change in level,

G29.6 (30-fold) and A165 (100-fold), are virtually constant throughout the normal temperature range, and are nearly invisible in autoradiograms of gels made from cells grown at these temperatures. Their behavior is presented in Fig. 4.

Three proteins, D74, G44, and G27.2, have levels that vary as linear functions of temperature over the entire range of 13.5 to 46°C (Fig. 5), despite the multiphasic effect of temperature upon cell growth over this temperature span (Fig. 1). These three "thermometer" proteins, plus 10 (B40.7, D49.2, E24.8, E42, F42.2, F74.5, G61, G127, I35.2, and I47.2) that show less than a 1.5-fold change at any temperature, are the major exceptions to the general rule that proteins vary little in the normal range (23 to 37°C) and vary significantly above and below this range.

Many of the proteins measured in this study were included in our recent report on the amounts of individual proteins during balanced growth at 37°C in five different media (17). In Fig. 6 we present all of the proteins for which both "metabolic regulation" and temperature response data are available. The left side of each panel shows the variation of proteins as a function of growth rate in different media at 37°C, and the right side of each panel shows the variation of these same proteins as a function of temperature in rich medium. Of the 133 proteins in the current study, 13 were not measured in our studies of metabolic regulation, and 10 exhibited individual and unique patterns of behavior in the five media and are therefore not displayed in Fig. 6. Of those remaining, 50 belong to three classes of metabolic regulation (Ia4, Ib, and Ic) that display a heterogeneous response to temperature; there is no way of predicting how a protein belonging to these three metabolic

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