movements caused an Apollo 14 g-jitter level on the order of 10^{-3} to $10^{-4}g$. An increase in heat transfer as the result of g-jitter was predicted by an analysis performed in 1961 by Gebhart (*8*). Earlier studies of vibration effects on gravity-driven convection (*9*) indicate that gravity tends to dampen the effects of vibrations. In low-g environments, therefore, vibrations will affect heat transfer processes more profoundly than they do on Earth.

Further details of these experiments and the data analyses are presented elsewhere (*10*).

PHILOMENA G. GRODZKA
Lockheed Missiles & Space Company, Inc., Huntsville, Alabama 35807
TOMMY C. BANNISTER
National Aeronautics and Space Administration, Marshall Space Flight Center, Alabama 35812

References and Notes

1. P. G. Grodzka and T. C. Bannister, *Science* **176**, 506 (1972).
2. J. R. A. Pearson, *J. Fluid Mech.* **4**, 489 (1958).
3. D. A. Nield, *ibid.* **19**, 341 (1964).
4. H. J. Palmer and J. C. Berg, *ibid.* **47**, 779 (1971).
5. L. E. Scriven and C. V. Sternling, *ibid.* **19**, 321 (1964); K. A. Smith, *ibid.* **24**, 401 (1966).
6. G. G. Hoard, C. R. Robertson, A. Acrivos, *Int. J. Heat Mass Transfer* **13**, 849 (1970).
7. E. L. Koschmieder, *J. Fluid Mech.* **30**, 9 (1967).
8. B. Gebhart, *Am. Inst. Aeronaut. Astronaut. J.* **1**, 380 (1963).
9. H. Y. Pak, E. R. F. Winter, R. J. Schoenals, in *Augmentation of Convective Heat and Mass Transfer*, A. E. Bergles and A. L. Wibb, Eds. (American Society of Mechanical Engineers, New York, 1970), p. 148.
10. T. C. Bannister, P. G. Grodzka, L. W. Spradley, S. V. Bourgeois, R. O. Hedden, B. R. Facemire, "Apollo 17 heat flow and convection experiments: Final data analyses results" [*NASA Publ. TM X-64772* (1973)].
11. We thank astronauts Ronald E. Evans (Apollo 17) and Stuart A. Roosa (Apollo 14) for their enthusiastic participation in the heat flow and convection experiments. Their careful performances of the experiments are in large measure responsible for the successes achieved. Supported by NASA contract NAS8-25577.

22 April 1974; revised 16 September 1974 ■

Troponin and Parvalbumin Calcium Binding Regions Predicted in Myosin Light Chain and T4 Lysozyme

Abstract. *A computer search of available protein sequences and structures suggests that bacteriophage T4 lysozyme contains one region and that rabbit myosin light chains contain three regions similar, and supposedly homologous, to the calcium binding region of carp muscle calcium binding parvalbumin.*

Each of the two calcium binding regions of carp muscle calcium binding parvalbumin (MCBP) consists of an α helix, a loop about the calcium ion, and another α helix (*1*), a so-called EF hand as in Fig. 1. Kretsinger (*2*) interpreted this similarity between the CD hand and the EF hand as having arisen from gene duplication and suggested that the calcium binding component of troponin (TN-C) consists of reduplicated forms of the EF hand. Collins *et al.* (*3*) recognized four homologous EF hands in the amino acid sequence of rabbit TN-C. This was consistent with the finding of Potter *et al.* (*4*) that there are four calcium ions bound per TN-C molecule. Kretsinger (*5*) then proposed (i) that the structure of TN-C consists of two pairs of EF hands arranged in approximate point group symmetry, 222; (ii) that many proteins involved in calcium controlled or mediated processes would be homologous and would consist of one or several EF hands; and (iii) that these EF hands could be recognized in protein sequences by considering which residues are most critical to the structures of the six (two in MCBP and four in TN-C) EF hands already recognized (Table 1).

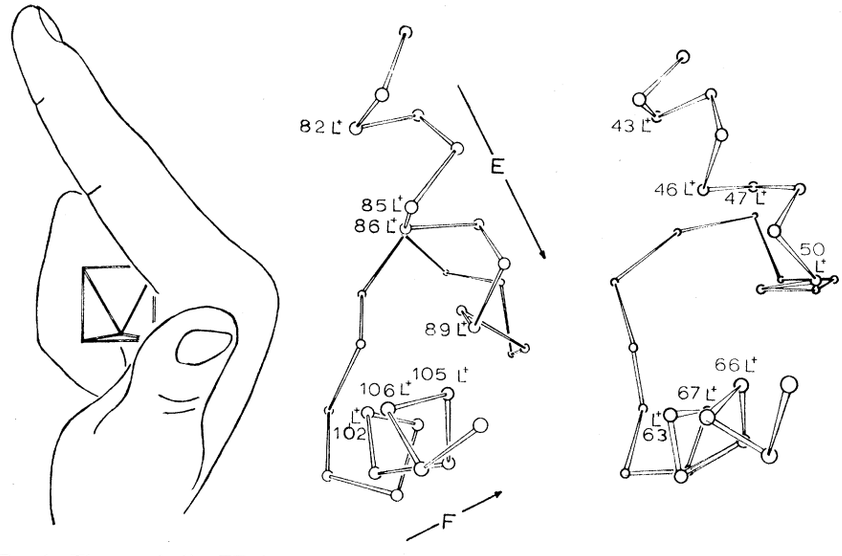
We (*6*) have written a series of computer programs to compare a given sequence or tertiary structure with a series of test sequences or structures. We examined all sequences available in the *Atlas of Protein Sequence and Structure* (*7*) and in its 1973 supplement as well as most of the more recently published ones. Where multiple sequences are available for one protein we used only one or two. We tested 136 different proteins containing 19857 amino acids, or 16049 stretches consisting of 29 residues each. A perfect alignment, as defined in the legend to Table 1, is 16. The overall distribution of alignment scores is:

0	1	2	3	4	5	6
661	1729	3320	3885	3186	1931	875

7	8	9	10	11	12	13	14	15	16
339	98	15	3	0	2	0	3	1	1

The six highest alignment scores, as expected, came from the six EF hands of TN-C and MCBP.

The next highest alignment scores—12, 10, and 10—came from three regions of the alkali extractable light chains (ALC–α,γ,δ) from rabbit skeletal myosin (*8*). Collins (*9*) using standard scoring schemes also recognized the ALC-α homology and sent us his results when we were completing our calculations. There is no evidence that ALC binds calcium, at least when they are detached from the myosin "hexamer" [two heavy chains, two nearly identical alkali light chains, and two light chains extractable with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNBLC)]. Nonetheless, it is not too surprising that ALC is homologous to the other muscle proteins—MCBP and TN-C. In molluscan muscle, calcium regulation is



Fg. 1. The symbolic EF hand on the left represents helix E (forefinger), the calcium binding loop (middle finger enclosing an octahedron), and helix F (thumb). The α-carbon skeletal models of the carp MCBP EF hand (center) and the T4 lysozyme EF hand (right) are superimposable to 1.9 Å.

Table 1. Comparison of EF hand sequences. The relevant sequences of the four proteins—calcium binding component 3 of carp muscle parvalbumin (MCBP), calcium binding component of troponin (TN-C), alkali light chain of rabbit skeletal muscle (ALC), and bacteriophage T4 lysozyme—are aligned by their correspondence to the test sequence of the first row. In the test sequence L* is a hydrophobic residue (L, leucine; I, isoleucine; V, valine; F, phenylalanine; M, methionine); D* has an oxygen atom as a calcium ligand (D, aspartic acid; N, asparagine, E, glutamic acid; Q, glutamine; S, serine; T, threonine); G (glycine) at residue 95, EF hand numbering, is at a sharp bend in the loop; I-97 attaches the loop to the hydrophobic core. Additional codings are R, arginine; H, histidine; Y, tyrosine; K, lysine. The vertical lines indicate the regions of the α helices which face the inside of the molecule (see Fig. 1). X,Y,Z,-Y,-X,-Z refer to vertices of the calcium coordination octahedron. In MCBP the oxygen ligand at the -Y vertex is provided by the peptide group; hence any amino acid at position 96 may provide a peptide oxygen for calcium coordination. The first column lists the alignment score for the test sequence of the first line. At each of the 16 positions where a *Test* residue exists, the sequence in question is scored 1 or 0 for the presence or absence of the test residues. $\bar{A}$ refers to the average of the Dayhoff (*7*) alignment scores for each region compared with the nine (inferred) EF hand regions of the muscle proteins. $\bar{A}$ is defined by Dayhoff as $(s - m)/\delta$, where s is an observed sequence alignment score; m is the mean alignment value for the same amino acid composition in random sequence; and δ is the standard deviation of the m scores. Lig and CO_2^- are the (inferred) number of oxygen-containing, calcium ligands and of those, carboxyl groups.

TEST	"Ā"	Lig	CO_2^-	REGION	SEQUENCE
				TEST	$_{1}$E L* L* L* D* D* D* G I D* E L* L* L* $_{29}$
14	3.2	6	4	MCBPCD	$_{38}$K S A D D V K K A F A I I D Q D K S G F I E E D E L K L F L Q N F K A D A R A$_{76}$
12	3.7	5	4	MCBPEF	$_{77}$L T D G E T K T F L K A G D S D G D G K I G V D E F T A L V K A$_{108}$
14	3.9	5	3	TN-C 1	$_{14}$E M I A E F K A A F D M F D A D G G G D I S V K E L G T V M R M L G Q T$_{49}$
16	3.9	6	4	TN-C 2	$_{50}$P T K E E L D A I I E E V D E D G S G T I D F E E F L V M V R Q M K E D A K G$_{88}$
14	4.0	6	4	TN-C 3	$_{89}$K S E E E L A E C F R I F D R N A D G Y I D A E E L A E I F R A S G E H$_{124}$
15	3.6	6	5	TN-C 4	$_{125}$V T D E E I E S L M K D G D K D N D G R I D F D E F L K M M E G V Q$_{158}$
12	3.6	6	2	ALC α	$_{46}$E Q Q D E F K E A F L L Y D R T G D S K I T L S Q V G D V L R A L G T N$_{81}$
10	2.6	4	3	ALC γ	$_{123}$G T Y E D F V E G L R V F D K E D G T V G M G A E L R H V L A T L G E$_{157}$
10	2.8	5	1	ALC δ	$_{158}$K M K E E E V E A L M A G Q E D S N G C I N Y E A F V K H I M S I$_{190}$
10	2.0	5	1	T4 LYS	$_{38}$S L N A A K S E L D K A I G R N C N G V I T K D E A E K L F N Q D V D A$_{73}$
				LIGANDS	X Y Z -Y -X -Z

exerted on the thick filament through a light chain extractable with ethylenediaminetetraacetic acid (EDTALC) (*10*). Kendrick-Jones (*11*) has shown that rabbit DTNBLC can restore calcium sensitivity to molluscan myosin whose EDTALC's have been extracted. We predict that all of these myosin light chains are homologs, and further that they can, at least under certain conditions, bind calcium as part of their regulatory function.

It was totally unexpected that the tenth highest score, 10/16, came from residues 42 through 70 of bacteriophage T4 lysozyme. At the time of this result we received from Matthews and Remington (*12*) a copy of their description of the crystal structure of T4 lysozyme. Region 42 through 70 is essentially superimposable on the EF hand of MCBP (Fig. 1). There is no published evidence that lysozyme binds calcium. Although they do not identify a calcium ion within the lysozyme EF loop, they do find the electron density in the region of residues 50 to 56 difficult to interpret. We predict that this loop does in fact coordinate an unanticipated calcium ion.

The prediction and verification of a structure resembling the EF hand in T4 lysozyme suggests three other considerations: Apparently EF hand resembling structures do not occur in globular proteins simply because they have high contents of α helices. We (*6*) have compared the tertiary structure of the EF hand with the structure available for 15 different (that is, myoglobin and hemoglobin are not "different") proteins. This comprised 2370 amino acids or 1950 comparisons of stretches 29 residues long. The minimum value of the residual,

$$R = \sum_{i=1}^{29} |\alpha_{\text{EF hand}_i} - \alpha_{\text{other}_i}|/29$$

Table 2. As in Table 1, $\bar{A}$ is the average of the Dayhoff alignment scores; Lig and CO_2^- would be the number of calcium ligands and carboxyl groups if these regions were calcium binding EF hands. Under 3-D we indicate whether the x-ray structure is known or can be inferred from homologs. None of the eight proteins of known or inferred structure contain EF hands at the indicated regions.

$\bar{A}$	Lig	CO_2^-	3-D	Protein	Residues
2.6	3	1		Tryptophan synthetase	215 243
2.4	5	1	X-ray	Adenylate kinase (pig)	165 193
1.9	5	2		Enterotoxin B*	20 48
1.9	5	1	Infer	Immunoglobulin M	130 158
1.6	4	1		Thioredoxin†	37 65
1.5	4	2	Infer	Troponin C (rabbit)	73 101
1.4	6	3		Flagellin‡	111 139
1.2	3	2	Infer	Troponin C (rabbit)	74 102
1.0	5	3		Prolactin (sheep)	100 128
0.9	3	0	Infer	Troponin C (rabbit)	38 66
0.8	4	1	X-ray	Concanavalin A	209 237
0.7	3	1	X-ray	Alcohol dehydrogenase	267 295
0.7	5	0		Fibrinogen gamma	56 84
0.4	4	1	Infer	Lactalbumin (cow)	71 99
0.4	4	1		Avidin (chicken)	91 119

* *Staphylococcus.* † *Escherichia coli.* ‡ *Bacillus subtilis.*

was 3.0 Å for carboxypeptidase, residues 263 through 295. This region is definitely not an EF hand. By contrast the R (for EF in comparison with lysozyme) is 1.9 Å.

In order to test the discriminating power of our sequence scoring scheme we have programmed six scoring schemes for the detection of homologies (*6*). For example, in Dayhoff's (*7*) scheme random sequences would generate the alignment score $A \geq 2.6$ once per hundred and $A \geq 3.3$ once per thousand comparisons. In Table 1 we list average A values for each of the nine regions of the muscle proteins compared with each other; they are detectable as homologs by such statistical tests. For T4 lysozyme compared with these nine regions, A ranges from 1.1 to 4.1 with $\overline{A} = 2.0$. This would hardly be considered significant. Tentatively we conclude that our special scoring scheme is marginally more sensitive. A more stringent evaluation will be provided by the 15 sequences that scored 9/16 on our test (Table 2). We do not suggest that these contain EF hands, even though their $\overline{A}$ values range from 0.4 to 2.6. Eight of these 15 regions definitely are not EF hands. This is seen in their x-ray structures or is inferred from structures of their homologs.

Concerning the question of possible T4 lysozyme homology with the muscle proteins—MCBP, TN-C, and ALC—Kretsinger (*5*) proposed that calcium control proteins are homologous. Conversely we might suspect that all proteins that contain an EF hand are involved in calcium mediated processes. There is no evidence that T4 lysozyme even binds calcium, let alone that it is regulated by calcium ions. However, there is really no evidence that either the alkali or the DTNBLC bind calcium either. It is possible that one of the postulated EF hands of ALC evolved from a calcium binding hand but subsequently lost its calcium binding affinity. For instance, the ALC-δ region has five oxygen atoms as potential calcium ligands but only one carboxyl group. This is also the case in T4 lysozyme. This argument, of a noncalcium binding EF hand, is weakened by the fact that in the three nonhomologous proteins—concanavalin A (*13, 14*), thermolysin (*15*), and *Staphylococcus* nuclease (*16*)—the six calcium ions are six coordinate with oxygen but the number of carboxyl groups ranges from one (*16*) to four (*17*). We suggest that T4 lysozyme derived from a calcium modulated enzyme of the host bacterium.

Alternatively, the EF hand to T4 lysozyme may not be homologous to those of the muscle proteins. It may be simply a thermodynamically preferred conformation which has been arrived at by an alternative evolutionary route. After all, the existence of a pair of α helices does not imply homology. The spatial relation of the T4 lysozyme E and F helices may be fortuitous, or the EF hand may have an inherent stability that is not yet understood.

In conclusion, the strong suggestion of three calcium binding EF hands in alkali light chains from rabbit skeletal muscle supports the theory of a common evolutionary origin for the calcium control proteins. The prediction and discovery of an EF hand in T4 lysozyme again poses the question of convergent versus divergent evolution at the molecular level (*18*).

ROBERT M. TUFTY
ROBERT H. KRETSINGER
Department of Biology, University of Virginia, Charlottesville 22901

References and Notes

1. R. H. Kretsinger and C. E. Nockolds, *J. Biol. Chem.* **248**, 3313 (1973).
2. R. H. Kretsinger, *Nat. New Biol.* **240**, 85 (1972).
3. J. H. Collins, J. D. Potter, M. J. Horn, G. Wilshire, N. Jackman, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **36**, 268 (1973).
4. J. D. Potter, J. C. Seidel, P. Leavis, S. S. Lehrer, J. Gergely, in *Symposium on Calcium Binding Proteins*, W. E. Drabikowski, Ed. (Elsevier, New York, 1974).
5. R. H. Kretsinger, in *Perspectives in Membrane Biology*, S. Estrada and C. Gitler, Eds. (Academic Press, New York, 1974).
6. R. M. Tufty and R. H. Kretsinger, in preparation.
7. M. O. Dayhoff, *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Washington, D.C., 1972).
8. G. Frank and A. G. Weeds, *Eur. J. Biochem.* **44**, 317 (1974).
9. J. H. Collins, *Biochem. Biophys. Res. Commun.* **58**, 301 (1974).
10. A. G. Szent-Gyorgyi, E. M. Szentkiralyi, J. Kendrick-Jones, *J. Mol. Biol.* **74**, 174 (1973).
11. J. Kendrick-Jones, *Nature (Lond.)* **249**, 631 (1974); R. D. Bremel, *ibid.* **252**, 405 (1974).
12. B. W. Matthews and S. J. Remington, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 4178 (1974).
13. G. M. Edelman, B. A. Cunningham, G. N. Reeke, J. W. Becker, M. J. Waxdal, J. L. Wang, *ibid.* **69**, 2580 (1972).
14. K. D. Hardman and C. F. Ainsworth, *Biochemistry* **11**, 4910 (1972).
15. B. W. Matthews, J. N. Jansonius, P. M. Colman, B. P. Schoenborn, D. Dupourque, *Nat. New Biol.* **238**, 37 (1972).
16. F. A. Cotton, C. J. Bier, V. W. Day, E. E. Hazen, S. Larson, *Cold Spring Harbor Symp. Quant. Biol.* **36**, 243 (1971).
17. We thank J. H. Collins as well as B. W. Matthews and S. J. Remington for sending us unpublished results and for subsequent discussions.
18. *Note added in proof*: (i) A. Weeds and A. McLachlan have kindly sent us [*Nature (Lond.)*, in press] a draft of their paper in which they have interpreted the ALC sequence in terms of four EF hands (1,2,3,4) as opposed to our interpretation of three (α,γ,δ). In order to align the β region with the EF hand they have effectively postulated that it contains three, single amino acid insertions. (ii) B. W. Matthews and S. J. Remington (*12*) have reinterpreted the T4 lysozyme electron density in the "loop" region, residues 50 to 56. Although the α helices of E and F remain unchanged, several of the loop atoms shift up to 5 Å. The revised lysozyme loop fits less well to the EF hand loop than does the structure used in their original publication. This revision seems to strengthen the interpretation of convergent evolution and to weaken the prediction of calcium binding and of homology.
19. Supported by NSF grant GB-29306 and a career development award to R.H.K.

12 August 1974; revised 16 September 1974

Daily Rhythm in Human Urinary Melatonin

Abstract. *The melatonin in urine samples from six healthy adult volunteers was concentrated on Amberlite XAD-2 resin, eluted with organic solvents, and quantitated by use of a bioassay technique (the dermal melanophore response of larval anurans to melatonin in their bathing medium). The melatonin content of samples collected between 11 p.m. and 7 a.m. was, in each case, several times higher than that of samples collected between 7 a.m. and 3 p.m. or between 3 p.m. and 11 p.m.*

Exogenous melatonin modifies sleep, locomotor activity rhythms, the electroencephalogram, the serotonin content of the brain, and, via a central action, secretion of pituitary gonadotropins (*1*). The rate at which the pineal organ normally synthesizes this hormone in vivo has been estimated indirectly by measuring pineal melatonin concentrations (*2*) and the in vitro activities of pineal enzymes that catalyze its biosynthesis (*3*). Data from such studies have been interpreted to show that melatonin synthesis in rats varies with a 24-hour rhythm, attaining maximum rates soon after the onset of the daily dark period. Studies on pineal constituents cannot, of course, be performed on material from human subjects. Thus, it has not been possible to determine whether melatonin synthesis also varies diurnally in human pineals and, if so, whether the rhythms in diurnally active humans and nocturnally active rats are in phase.

Barchas and Lerner (*4*), using a countercurrent isolation technique and a frog skin bioassay, demonstrated that melatonin could be detected in a 48-hour specimen of human urine. While