References and Notes

1. J. A. Burns, *Rev. Geophys. Space Phys.* **10**, 463 (1972).
2. J. B. Pollack, J. Veverka, M. Noland, C. Sagan, T. C. Duxbury, C. H. Acton, Jr., G. H. Born, W. K. Hartmann, B. A. Smith, *J. Geophys. Res.* **78**, 4313 (1973).
3. B. H. Zellner and R. C. Capen, *Icarus* **23**, 437 (1974).
4. K. D. Pang, J. B. Pollack, J. Veverka, A. L. Lane, J. M. Ajello, *Science* **199**, 64 (1978).
5. F. O. Huck, H. F. McCall, W. R. Patterson, G. R. Taylor, *Space Sci. Instrum.* **1**, 189 (1975).
6. J. B. Pollack, D. Colburn, R. Kahn, J. Hunter, W. Van Camp, C. W. Carlston, M. R. Wolfe, *J. Geophys. Res.* **82**, 4479 (1977).
7. G. P. Kuiper as quoted by D. L. Harris, in *Planets and Satellites*, G. P. Kuiper and B. M. Middlehurst, Eds. (Univ. of Chicago Press, Chicago, 1961), p. 272.
8. M. J. Gaffey, *J. Geophys. Res.* **81**, 905 (1976).
9. J. Gros *et al.*, in *Proceedings of the Seventh Lunar Science Conference* (Pergamon, New York, 1976), p. 2403.
10. R. H. Tolson *et al.*, *Science* **199**, 61 (1978).
11. S. F. Singer, in *The Physical Studies of Minor Planets*, T. Gehrels, Ed. (NASA publication SP-267, National Aeronautics and Space Administration, Washington, D.C., 1971).
12. C. R. Chapman, D. Morrison, B. Zellner, *Icarus* **25**, 104 (1975); D. Morrison, *ibid.* **31**, 185 (1977).
13. J. S. Lewis, *Earth Planet. Sci. Lett.* **15**, 286 (1972); *Science* **186**, 440 (1974).
14. J. B. Pollack, J. Burns, M. Tauber, *Bull. Am. Astron. Soc.* **9**, 455 (1977).
15. D. Hunten, personal communication.

16. We thank the members of the Viking lander imaging team for their help in obtaining the photographs of Phobos, R. Kahn for his help in planning the pictures, and B. Baldwin for her aid in preparing the figures.

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Genetic Defect in Biosynthesis of the Precursor Form of the Fourth Component of Complement

Abstract. *Under cell-free conditions, liver polysomes from guinea pigs genetically deficient in the fourth component of complement (C4) did not synthesize pro-C4 (the precursor of C4), but did synthesize nascent C4 polypeptides which remained polysome bound. The defect was specific for pro-C4 synthesis since the amounts of total protein and albumin synthesis and release from C4-deficient polysomes were similar to that in normal guinea pig liver polysomes.*

Complement, a group of 19 plasma proteins, is one of the principal humoral effectors of inflammatory reactions. A deficiency of the fourth component of complement (C4) in guinea pigs, described by Ellman *et al.* (*1*), is inherited as an autosomal recessive trait and is genetically linked to histocompatibility loci (*2*). No C4 protein is found in serums from homozygous-deficient animals even with the use of sensitive methods capable of detecting one-millionth the normal levels of C4 (*3*). Cells and tissues from homozygous C4-deficient animals neither synthesize nor secrete C4 in vitro under conditions that support synthesis by normal cells (*4*). Somatic cell hybrids of C4-deficient peritoneal macrophages from guinea pigs with (human) HeLa cells synthesize C4 of human origin (*5*), although neither parent cell alone produces C4. A factor has been isolated from guinea pig peritoneal macrophages which is capable of inducing HeLa cells to synthesize and secrete C4 (*6*). The factor is present in C4-deficient macrophages at concentrations at least five times greater than that in normal macrophages. In order to investigate further the molecular basis for genetic control of C4 synthesis, we designed a cell-free protein synthesizing system (*7*). We now find, using this method, evidence for a defect in specific C4 messenger RNA (mRNA) translation on polysomes from homozygous C4-deficient guinea pigs.

Polysome preparations from a homogenate of normal guinea pig liver (S-20) (*8*) were incubated in the presence of radioactively labeled amino acids and an energy-generating system under optimal ionic conditions (*7*). Radioactively labeled C4 was detected by immunoprecipitation with C4 carrier protein and antiserum to guinea pig C4. Equivalent amounts of bovine gamma globulin (BGG) and antiserum to BGG or ovalbumin (OVA) and antiserum to OVA immunoprecipitates served as controls for nonspecific precipitation of radioactivity. Labeled guinea pig albumin was assayed in a similar manner with the use of guinea pig albumin (GPA) carrier protein and antiserum to GPA; controls consisted of equivalent precipitates of porcine albumin and antiserum to porcine albumin. In an earlier study, analysis of immunoprecipitates on sodium dodecyl sulfate (SDS) polyacrylamide gels indicated that C4 is synthesized as a single polypeptide chain precursor protein, designated pro-C4 (*7*), which is then converted to the three-chain structure (molecular weights of 95,000, 78,000, and 31,000) characteristic of serum C4 (*9*).

With these methods, the protein synthesizing capacities of normal and C4-deficient polysomes were compared. Polysomes from normal guinea pig liver and, in a separate reaction mixture, polysomes from homozygous C4-deficient guinea pigs were incubated for 90 minutes under optimal conditions for cell-free protein synthesis (*7, 8*). Polysomes were removed by centrifugation at 100,000*g* for 2 hours, and the released proteins were assayed for pro-C4 and GPA by immunoprecipitation. Control immunoprecipitates were also formed, and the radioactivity nonspecifically precipitated was compared to that in antiserum to C4 and antiserum to GPA precipitates. Total protein synthesis as assessed by precipitation of radioactivity with hot trichloroacetic acid (TCA) was similar in each. About 50 percent of the synthesized protein was released from polysomes, 3 to 4 percent of which was GPA and about 0.2 percent pro-C4. For C4-deficient polysomes, radioactivity specifically precipitated by antiserum to C4 was similar to control immunoprecipitates (Fig. 1). Analysis of the immunoprecipitates by means of electrophoresis on SDS-polyacrylamide gel (Fig. 1) showed synthesis and release of pro-C4 by normal polysomes, but no pro-C4 in the polysome-free supernatant from C4-

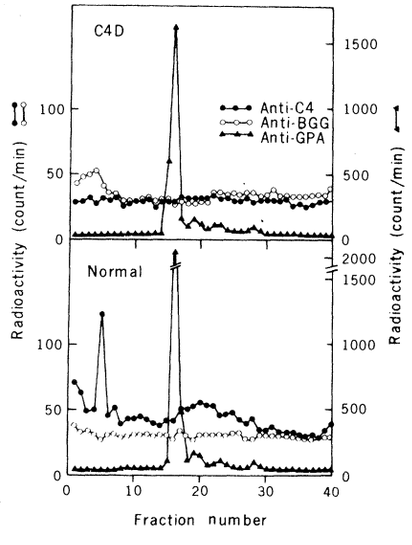
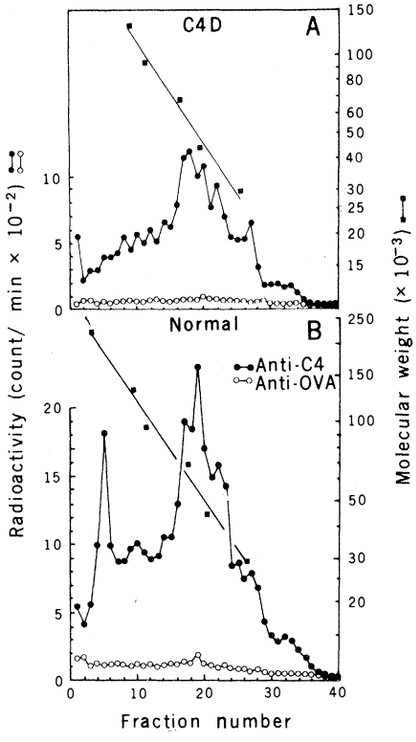


Fig. 1. Cell-free synthesis and release of pro-C4 and albumin by polysomes from homozygous C4-deficient (*C4D*) and normal guinea pig liver (*8*). Immunoprecipitable radioactivity was determined by electrophoresis on 1 percent SDS-polyacrylamide gel (5.6 percent) containing dithiothreitol (50 m*M*). Total and immunoprecipitable radioactivity (before application to the gels) is expressed as counts per minute per 100 μl. Normal: total protein (TCA) precipitate), 1.69×10^6; released from polysomes (TCA precipitate), 9.4×10^5; albumin, 4.12×10^4; C4, 3.49×10^3; BGG control, 1.68×10^3; porcine albumin control, 2.68×10^3. C4-deficient: total protein (TCA precipitate), 2.41×10^6; released from polysomes, 1.20×10^6; albumin, 3.56×10^4; C4, 1.12×10^3; BGG control, 1.00×10^3; porcine albumin control, 4.02×10^3. This experiment was repeated more than 12 times with normal S-20 and four times with homozygous C4-deficient S-20. In the normal S-20, radioactivity in the pro-C4 ranged from 100 to 2500 count/min (see also Fig. 2); no pro-C4 was detected in any of the C4-deficient S-20 preparations.



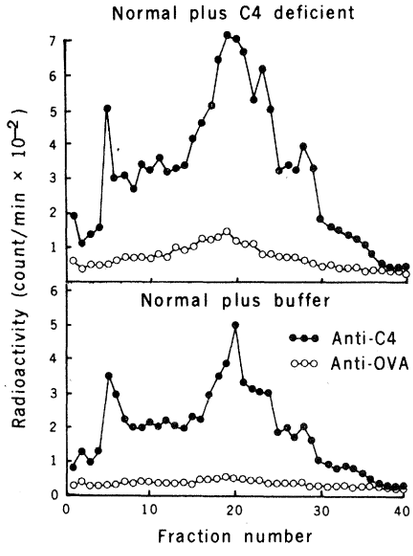


Fig. 2 (left). Synthesis of pro-C4 in (A) homozygous C4-deficient and (B) normal cell-free mixtures. Released protein plus polysome-bound nascent polypeptides were determined by electrophoresis on 1 percent SDS—polyacrylamide gel (5.6 percent) in dithiothreitol (50 m*M*). Total and immunoprecipitable radioactivity is expressed as counts per minute per 100 μl. Normal: total protein, 3.15×10^6; C4, 3.45×10^4; OVA control, 4.6×10^3. C4-deficient: total protein, 2.34×10^6; C4, 1.58×10^4; OVA control, 2.2×10^3. Fig. 3 (right). Mixture of polysomes from homozygous C4-deficient and normal guinea pig livers. Released pro-C4 plus polysome-bound nascent chains were determined by electrophoresis on 1 percent SDS polyacrylamide gel (5.6 percent) in dithiothreitol (50 m*M*).

deficient incubation mixtures. The amount of albumin synthesized was similar in both normal and C4-deficient mixtures, demonstrating the specificity of the defect in the synthesis of pro-C4. Radioactivity in control immunoprecipitates (BGG and antiserum to BGG) distributed uniformly throughout the gels.

Total cell-free incubation mixtures, containing released and polysome-bound protein, were also analyzed. Under these conditions, of the total protein in normal cell-free incubation mixtures about 0.9 percent was C4 antigen, whereas in the C4-deficient mixtures 0.6 percent was C4 antigen. Electrophoresis of these immunoprecipitates on SDS-polyacrylamide gel is shown in Fig. 2. The normal mixtures contained pro-C4 plus a heterogeneous mixture of nascent C4 peptides with molecular weights of 20,000 to 90,000. The C4-deficient mixtures contained no intact pro-C4, but a similar mixture of nascent C4 peptides bound to polysomes. The radioactivity in control, antiserum to OVA immunoprecipitates distributed uniformly without recognizable peaks of activity.

Normal guinea pig liver incubated in tissue culture with radioactively labeled amino acids secretes a C4 that is identical in size and subunit structure to serum C4 (*9*) and contains intracellular pro-C4 (*7*). No radioactivity was precipitated with antiserum in short-term tissue cultures of C4-deficient guinea pig liver, either in the medium or in tissue homogenates (data not shown), confirming earlier observations (*4*) with these more sensitive methods.

The detection of nascent peptides of C4 synthesized by polysomes from C4-deficient liver suggested the presence in the homozygous-deficient animal of a defective mRNA for C4, or a normal C4 mRNA that is defectively translated. Alternatively, the C4-deficient S-20 might contain a protease which degraded synthesized pro-C4. To examine the latter possibility, a mixture of normal S-20 and C4-deficient S-20 was incubated under cell-free conditions. Total protein synthesis was similar for normal S-20 (2.3×10^6 count/min per 100 μl) and the mixture of normal with C4-deficient S-20 (2.1×10^6 count/min per 100 μl). Total reaction mixtures (before removal of polysomes) were immunoprecipitated with antiserum to C4 and C4 carrier protein and applied to SDS-polyacrylamide gels (Fig. 3). In order to compare directly the normal S-20 and normal C4-deficient mixture, the normal S-20 cell-free products were diluted by half before immunoprecipitation. The amount of synthesized pro-C4 was approximately proportional to the amount of normal S-20 in the incubation mixture and was not decreased by incubation with C4-deficient S-20. Other mixtures with ratios of normal to C4-deficient S-20 from 9 to 0.11 have also been analyzed; the amount of pro-C4 synthesized correlated with the amount of normal S-20 in the incubation mixture (data not shown). These mixing experiments, therefore, make it unlikely that a protease accounted for the failure to detect pro-C4 in C4-deficient mixtures but do not rule out the possibility of an unstable pro-C4.

From these data we conclude that genetic deficiency of C4 in guinea pigs is probably the result of either a defective C4 mRNA or defective translation of normal C4 mRNA. This implies that in the C4-deficient guinea pig genome, at least part of the structural gene for C4 is present in a mutant form. Deficiency of C4 in guinea pigs may, therefore, be analogous to some types of β thalassemia in humans, in which both nonfunctional β-globin mRNA and defective translation of functional mRNA have been demonstrated (*10*).

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References and Notes

1. L. Ellman, I. Green, M. M. Frank, *Science* **170**, 74 (1970).
2. E. M. Shevach, M. M. Frank, I. Green, *Immunogenetics* **3**, 595 (1976).
3. M. M. Frank, J. May, T. Gaither, L. Ellman, *J. Exp. Med.* **134**, 176 (1971); L. Ellman, I. Green, F. Judge, M. M. Frank, *ibid.*, p.162.
4. H. R. Colten and M. M. Frank, *Immunology* **22**, 991 (1972).
5. H. R. Colten and R. Parkman, *Science* **176**, 1029 (1972).
6. H. R. Colten, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2233 (1972).
7. R. E. Hall and H. R. Colten, *ibid.* **74**, 1707 (1977).
8. The S-20 fractions of liver homogenates from normal and homozygous C4-deficient guinea pigs were prepared as described in (*7*) and incubated (90 minutes; 25°C) in the presence of 5 m*M* $MgCl_2$, 100 m*M* KCl, 2 m*M* adenosine triphosphate, 0.5 m*M* guanosine triphosphate, 15 m*M* phosphocreatine, creatine phosphokinase (10 μg/ml), 40 μ*M* concentrations of 19 amino acids excluding leucine, 10 μ*M* [^{3}H]leucine (500 μc/ml), 1.8 m*M* reduced glutathione, and 30 m*M* Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonate] (*p*H 7.3). After incubation, cell-free mixtures were diluted 1:3 with detergent (0.5 percent Triton-deoxycholate) buffer and centrifuged at 100,000*g* for 2 hours, after which specific and control immunoprecipitates were performed in 100-μl portions of the supernatant.
9. R. D. Schreiber and H. J. Muller-Eberhard, *J. Exp. Med.* **140**, 1324 (1974); R. E. Hall and H. R. Colten, *J. Immunol.* **118**, 1903 (1977).
10. B. G. Forget, *CRC Crit. Rev. Biochem.* **2**, 311 (1974); F. Conconi, P. T. Rowley, L. Del Senno, S. Pontremoli, S. Volpato, *Nature (London) New Biol.* **238**, 83 (1972); Y. W. Kan, J. P. Holland, A. M. Dorzy, H. E. Varmus, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 5140 (1975).
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