tion of LCAT (*2*). Thus, all of the lipoprotein abnormalities observed could be due to LCAT deficiency. There is evidence that lipoproteins secreted by the liver are different from those normally found in plasma and that the secreted forms are rapidly metabolized and transformed in the blood (*15*). The abnormal lipoproteins observed in LCAT deficiency might more nearly represent the secretory forms of the lipoproteins.

Lipoprotein classes have been studied in great detail in human familial LCAT deficiency (*2*). In these studies, VLDL, LDL, and HDL have all been shown to differ from normal both in lipid composition and in morphology. In this disease the LDL and HDL contain high concentrations of phospholipids and low concentrations of esterified cholesterol. It has been suggested that cholesteryl esters are necessary in order to maintain the normal, spherical structure of these lipoproteins and that, in their absence, bilamellar discoidal structures similar to phospholipid bilayers are formed.

The lipoproteins from the plasma of rats made LCAT-deficient by treatment with D-galactosamine exhibit an ultrastructural appearance essentially identical to that observed in familial LCAT deficiency. Furthermore, both the absence of electrophoretically demonstrable α-lipoprotein and the changes in lipid composition are similar to those observed in the human condition. Similar bilamellar stacked disks have also been described in liver disease. D-Galactosamine provides an experimental model with which to study the relationship of LCAT deficiency to lipoprotein abnormalities in rats. All of the changes are reversible and can be followed as they develop over a relatively short time period.

SEYMOUR M. SABESIN
LOIS B. KUIKEN
JAMES B. RAGLAND
Division of Gastroenterology, Department of Medicine, University of Tennessee College of Medicine, Memphis 38163

References and Notes

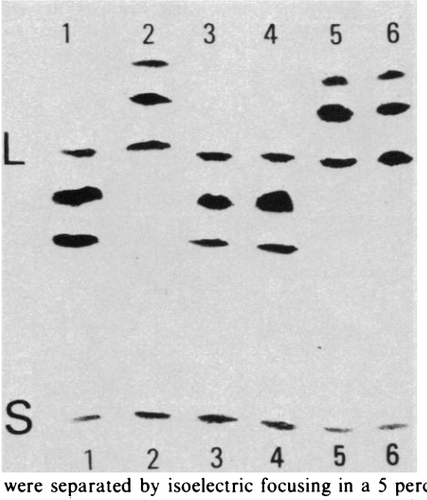
1. J. Glomset, in *Blood Lipids and Lipoproteins*, G. Nelson, Ed. (Wiley, New York, 1972), pp. 745 787.
2. J. A. Glomset, K. R. Norum, A. V. Nichols, T. Forte, W. C. King, J. Albers, C. D. Mitchell, K. R. Applegate, E. Gjone, *Scand. J. Clin. Lab. Invest.* **33** (Suppl. 137), 165 (1974); P. Alaupovic, W. J. McConathy, M. D. Curry, H. N. Magnani, H. Torsvik, K. Berg, E. Gjone, *ibid.*, p. 83; T. Forte, A. Nichols, J. Glomset, K. Norum, *ibid.*, p. 121.
3. E. Gjone and K. R. Norum, *Acta Med. Scand.* **187**, 153 (1970); R. L. Hamilton, R. J. Havel, J. P. Kane, A. E. Baluroch, T. Sata, *Science* **172**, 475 (1971); J. R. Blomhoff, S. Skede, S. Ritland, E. Gjone, *Scand. J. Gastroenterol.* **8** (Suppl. 20), 51 (1973); J. B. Simon, D. L. Kepkay, R. Poon, *Gastroenterology* **66**, 539 (1974); S. Calandra, M. J. Martin, N. McIntyre, *Eur. J. Clin. Invest.* **1**, 352 (1971).
4. A. M. Scanu, L. P. Aggerbeck, A. W. Kruski, C. T. Lim, H. J. Kayden, *J. Clin. Invest.* **53**, 440 (1974).
5. P. Clifton-Bligh, P. J. Nestel, H. M. Whyte, *N. Engl. J. Med.* **286**, 567 (1972); R. Scherer and G. Rubenstrath-Bauer, *Klin. Wochenschr.* **21**, 1059 (1973).
6. K. Decker and D. Keppler, in *Progress in Liver Disease*, H. Popper and F. Schaffner, Eds. (Grune & Stratton, New York, 1972), vol 4, pp. 183-199.
7. R. S. Koff, G. Gordon, S. M. Sabesin, *Proc. Soc. Exp. Biol. Med.* **137**, 696 (1971); R. S. Koff, J. J. Fitts, S. M. Sabesin, *ibid.* **138**, 89 (1971).
8. R. Katterman and D. I. Wolfrum, *Z. Klin. Chem.* **8**, 413 (1970).
9. K. T. Stokke and K. R. Norum, *Biochim. Biophys. Acta* **210**, 202 (1970).
10. J. P. Blomhoff, *Clin. Chim. Acta* **43**, 257 (1973).
11. The apparatus, reagents, and method used were purchased from Corning-ACI, Palo Alto, Calif. 94306.
12. F. T. Hatch and R. S. Lees, *Adv. Lipid Res.* **6**, 1 (1968).
13. J. B. Swaney, H. Reese, H. A. Eder, *Biochem. Biophys. Res. Commun.* **59**, 513 (1974).
14. C. J. Fielding, V. G. Shore, P. E. Fielding, *ibid.* **46**, 1493 (1972).
15. S.-P. Noel and D. Rubinstein, *J. Lipid Res.* **15**, 301 (1974); J. B. Marsh, *ibid.*, p. 544.
16. Supported by NIAMDD research grant 17398 and grants-in-aid from the American Heart Association, Tennessee Heart Association, and Veterans Administration. We thank B. Mays, V. Andersen, C. Hepner, and S. Frase for their technical assistance.

30 June 1975; revised 29 September 1975

Fraction 1 Protein and the Origin of Polyploid Wheats

Abstract. *The nature of the interspecific hybridizations giving rise to the polyploid wheats has been determined from an analysis of the polypeptide compositions of fraction 1 proteins isolated from diploid, tetraploid, and hexaploid wheat species. In the origins of the tetraploid wheat, the B-genome donor provided the chloroplast genome and was therefore the maternal parent in the cross with* Triticum monococcum. *Subsequently, the tetraploid wheat,* T. dicoccum, *was the maternal parent in the cross with* Aegilops squarrosa, *giving rise to the hexaploid wheat,* T. aestivum. *Of the small number of diploid species examined, only* Ae. speltoides *has a chloroplast genome similar to that of the polyploid wheats.*

The polypeptide composition of fraction 1 protein, as revealed by isoelectric focusing in the presence of 8*M* urea (*1*), provides excellent phenotypic markers for both the nuclear and chloroplast genomes of higher plants, because the genetic information for the primary structure of the small subunit is located in the nuclear genome (*2*), whereas the information for the large subunit is located in the chloroplast genome (*3*). In F_1 interspecific hybrids in the genus *Nicotiana*, fraction 1 protein is composed of large subunit polypeptides inherited solely from the maternal parent species and small subunit polypeptides inherited from both parent species (*4*). Doubling the chromosome number of such F_1 interspecific hybrids, giving rise to new self-fertile amphiploid species, does not affect the polypeptide composition of the fraction 1 protein (*5*). Therefore, examination of the polypeptide patterns of fraction 1 proteins isolated from amphiploid species and from their putative progenitor species provides a method for determining the origins of amphiploid species. This method has been used successfully to determine the identity of the interspecific hybrid which gave rise to the commercial tobacco plant, *N. tabacum* (*6*). To demonstrate the potential of



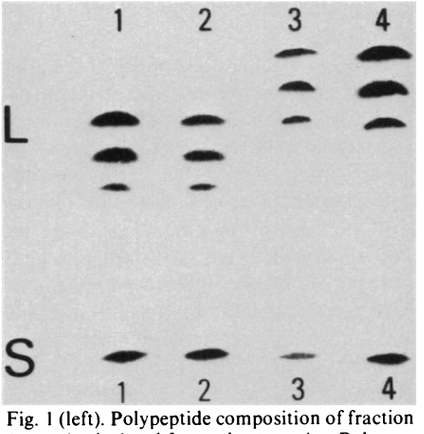


Fig. 1 (left). Polypeptide composition of fraction 1 proteins isolated from wheat species. Polypeptides of *S*-carboxymethylated fraction 1 proteins were separated by isoelectric focusing in a 5 percent polyacrylamide gel slab containing 1 percent ampholine, *p*H 5 to 7, and 8*M* urea. Polypeptide bands were stained with bromophenol blue. Samples (20 μg of protein) from left to right: (1) *Ae. squarrosa*; (2) *Ae. speltoides*; (3) *T. urartu*; (4) *T. monococcum*; (5) *T. dicoccum*; (6) *T. aestivum*. *L*, large subunit polypeptides, *p*H 5.8 to 6.1; *S*, small subunit polypeptides, *p*H 5.5. Fig. 2 (right). Polypeptide composition of fraction 1 protein from reciprocal, interspecific F_1 hybrids of *T. boeoticum* and *T. dicoccoides*. Polypeptides were separated as described in the legend to Fig. 1. Samples (20 μg of protein) from left to right: (1) *T. boeoticum*; (2) *T. boeoticum* ♀ × *T. dicoccoides* ♂; (3) *T. dicoccoides* ♀ × *T. boeoticum* ♂; (4) *T. dicoccoides*. *L*, large subunit polypeptides; *S*, small subunit polypeptides.

this method outside the genus *Nicotiana* we have examined the fraction 1 proteins of species implicated in the origins of polyploid wheat. These analyses provide some indications as to the nature of the B-genome donor and further indicate the directions of the interspecific crosses that gave rise to the tetraploid and hexaploid wheats.

The wheat species examined were the hexaploid *Triticum aestivum*, the tetraploids *T. timopheevii* and *T. dicoccoides* and its cultivated form *T. dicoccum*, and the diploids *Aegilops speltoides*, *Ae. squarrosa*, *T. urartu*, and *T. boeoticum* and its cultivated form *T. monococcum*. Fraction 1 proteins were isolated from the supernatant fraction obtained by homogenizing 14-day-old leaves in 3 volumes of 0.05*M* tris-HCl buffer, *p*H 7.8, containing 0.2*M* NaCl, 0.5 m*M* ethylenediaminetetraacetic acid, 10 m*M* potassium metabisulfite, and 20 percent (weight to volume) Dowex-1(×2) and then centrifuging for 60 minutes at 77,000*g*. Fraction 1 proteins were precipitated from the extract by ammonium sulfate at 50 percent saturation, and then extraneous proteins were removed by passage through a column of Sepharose 4B to which antibodies to fraction 1 protein had been covalently linked (*7*). Fraction 1 protein bound to the column was then eluted in the form of dissociated subunits by 8*M* urea in 0.05*M* tris-HCl, *p*H 7.8, 0.2*M* NaCl. The protein was then *S*-carboxymethylated before analysis by isoelectric focusing in the presence of 8*M* urea (*1*).

As shown in Fig. 1, all fraction 1 proteins from the wheat species contain three large subunit polypeptides and a single small subunit polypeptide. The species fall into two groups on the basis of the isoelectric points of the large subunits. One group, having higher isoelectric points, contains *T. aestivum*, *T. dicoccum*, and *Ae. speltoides*, as well as *T. timopheevii* (not shown) and *T. dicoccoides* (see Fig. 2). The other group, with lower isoelectric points for the large subunit polypeptides, contains the diploid species, *T. boeoticum*, *T. urartu*, and *Ae. squarrosa*, as well as *T. monococcum* (not shown). These differences allow some conclusions to be drawn concerning the course of evolution of the polyploid wheats, because the mode of inheritance of the large subunit polypeptides of fraction 1 protein is the same in wheat as in the *Nicotiana* species. This is shown in Fig. 2 by an analysis of the polypeptide composition of fraction 1 proteins from reciprocal interspecific hybrids of *T. boeoticum* and *T. dicoccoides*. The large subunit polypeptides of fraction 1 protein from the hybrid species are inherited solely from the maternal parent, as has been demonstrated previously for interspecific hybrids in the genus *Nicotiana* (*4*).

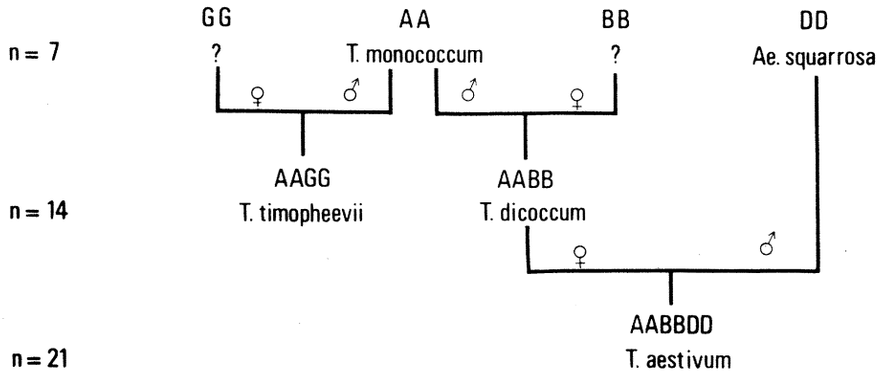


Fig. 3. Diagram of the origins of polyploid wheat species determined from the polypeptide composition of fraction 1 proteins.

Conclusions concerning the direction of the crosses giving rise to the polyploid wheats can therefore be drawn from the isoelectric focusing patterns shown in Fig. 1. The large subunit pattern of fraction 1 protein from the hexaploid wheat, *T. aestivum* (AABBDD), which is recognized on the basis of cytogenetic evidence to have arisen by hybridization of *T. dicoccum* (AABB) and *Ae. squarrosa* (DD) (*8*), could have been produced from these two species only if the direction of the cross were female *T. dicoccum* × male *Ae. squarrosa*. Similarly, the directions of the hybridizations giving rise to the tetraploid wheats, *T. dicoccum* and *T. timopheevii*, can be determined, even though in each case there is some controversy over the identity of the diploid donor of one of the genomes. *Triticum monococcum* is established as the donor of the A genome to both of the tetraploid wheats (*9*), and the present analysis indicates that *T. monococcum* could not have provided the genetic information for the large subunit polypeptides of the fraction 1 protein of either *T. dicoccum* or *T. timopheevii*. This suggests that in the origin of *T. dicoccum*, the B-genome donor was also the source of the chloroplast genome and must therefore have been the maternal parent. Similarly, the donor of the other (G) genome to *T. timopheevii* must have been the maternal parent in the cross with *T. monococcum*. These relationships are presented in Fig. 3.

Of the diploid species examined, only *Ae. speltoides* has the same large subunit pattern as *T. dicoccum* and *T. timopheevii* and is therefore the only species examined that could have provided the chloroplast genome of the polyploid wheats. On the basis of cytogenetic evidence *Ae. speltoides* has recently been suggested as the source of the other (G) genome in *T. timopheevii* (*10*). However, the identification of *Ae. speltoides* as the B-genome donor is a matter of contention. Although *Ae. speltoides* was accepted as the B-genome donor on the basis of morphological (*11*) and cytogenetic (*12*) evidence accumulated over a period of 30 years, more recent evidence on chromosome pairing (*13*) and the electrophoretic analysis of seed proteins (*14*) have cast considerable doubts on the involvement of *Ae. speltoides* in the origin of the emmer wheats. It has recently been suggested that *T. urartu* fulfills most of the criteria necessary to identify the B-genome donor (*15*), but the present study indicates that *T. urartu* could not have provided the genetic information for the large subunit pattern of *T. dicoccum*. However, it must be pointed out that the plant material analyzed was grown from single accessions of each of the species and it is possible that variation of the polypeptide composition of fraction 1 protein occurs within species. Although no such variation has been encountered in numerous collections of *Nicotiana* species (*16*), Kihara (*17*) has demonstrated by hybridization experiments that two different cytoplasms occur in varieties of the diploid species *Ae. caudata*. If a similar situation were to exist in other diploid species, then isoelectric focusing of fraction 1 protein, in conjunction with cytogenetic and electrophoretic studies, might lead to the identification of plants with the properties expected of the B-genome donor.

K. CHEN, J. C. GRAY*, S. G. WILDMAN
Department of Biology and *Molecular Biology Institute, University of California, Los Angeles 90024*

References and Notes

1. S. D. Kung, K. Sakano, S. G. Wildman, *Biochim. Biophys. Acta* **365**, 138 (1974).
2. N. Kawashima and S. G. Wildman, *ibid.* **262**, 42 (1972).
3. P. H. Chan and S. G. Wildman, *ibid.* **277**, 677 (1972).
4. K. Sakano, S. D. Kung, S. G. Wildman, *Mol. Gen. Genet.* **130**, 90 (1974).
5. S. D. Kung *et al.*, *J. Mol. Evol.*, in press.
6. J. C. Gray, S. D. Kung, S. G. Wildman, S. J. Sheen, *Nature (Lond.)* **252**, 226 (1974).
7. J. C. Gray and S. G. Wildman, *Plant Sci. Lett.*, in press.
8. E. S. McFadden and E. R. Sears, *Rec. Genet. Soc. Am.* **13**, 26 (1944); R. Riley and V. Chapman, *Wheat Inform. Serv.* **11**, 18 (1960).
9. H. Kihara, *Bot. Mag. (Tokyo)* **33**, 17 (1919); K. Sax, *Genetics* 7, 513 (1922); F. Lilienfeld and H. Kihara, *Cytologia* **6**, 87 (1934).

10. H. L. Shands and G. Kimber, *Proc. 4th Int. Wheat Genet. Symp.* (1973), p. 101.
→ P. Sarkar and G. L. Stebbins, *Am. J. Bot.* **43**, 297 (1956).
12. R. Riley, J. Unrau, V. Chapman, *J. Hered.* **49**, 90 (1958).
13. G. Kimber and R. S. Athwal, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 912 (1972).
→ B. L. Johnson, *ibid.*, p. 1398.
15. ———, *Can. J. Genet. Cytol.* **17**, 21 (1975).
16. K. Chen, S. D. Kung, J. C. Gray, S. G. Wildman, *Biochem. Genet.*, in press.
17. H. Kihara, *Proc. 3rd Int. Wheat Genet. Symp.* (1968), p. 125.
18. Supported by NSF grant 75-07368 and AEC contract AT(04-3)-34, project 8. We are grateful to Drs. B. L. Johnson and H. S. Dhaliwal of the Department of Plant Sciences, University of California, Riverside, for helpful discussions and for supplying the plant material.
* Present address: Department of Biochemistry, University of Cambridge, Cambridge, England.

20 August 1975

Transferrin: Internal Homology in the Amino Acid Sequence

Abstract. *Two regions of the primary structure of human serum transferrin, of 87 and 57 residues, are reported. When these are suitably aligned by placing two gaps, 40 percent of the amino acids in corresponding positions are identical. This indicates that the doubling of an ancestral structural gene occurred during the evolution of the transferrins.*

Serum transferrin, which functions in the transport of ferric iron in the circulation, consists of a single glycopolypeptide chain of molecular weight 77,000 (*1*), within which are two similar binding sites for Fe^{3+} and other metals (*2*). Similar proteins are found in avian egg whites (ovotransferrins) and in milk and other secretions (lactoferrins), which probably have an antimicrobial function. The similar nature of the metal binding sites, both of which require concomitant binding of an anion with the metal (*3*), and the large size of the polypeptide chain have been taken by some to suggest that the protein may have originated during phylogeny by the doubling in size of the structural gene for a smaller precursor (*1, 4*). This history would be expected to be evident in the amino acid sequence of a modern transferrin as regions of internal homology, but previous structural studies of human transferrin (*5*) and human lactoferrin (*6*) have not provided any convincing evidence for internal homology. In the sequences around 34 unique cystinyl residues in ovotransferrin, Elleman and Williams (*7*) found signs of similarity only in two peptides of nine residues each, while Graham and Williams found no internal homology between sequences around the two glycosylation sites in human and other transferrins (*8*).

Studies by Williams have, however, shown that fragments of approximately half the molecular weight of the intact protein can be obtained from ovotransferrin by limited proteolysis (*9*). These fragments appear to correspond to the amino and carboxy terminal halves of the polypeptide chain, and each contains an intact Fe^{3+} binding site (*9*). This observation is consistent with the presence of two largely independent conformational domains within the transferrin molecule, each associated with one of the metal binding sites. A structure of this type might well be expected to be associated with internal homology.

In our previous structural studies with human serum transferrin, we have isolated and characterized the nine fragments obtained by CNBr cleavage at the eight methionyl residues (*10*). The sizes of these fragments (designated CN-1 to CN-9) account for the whole polypeptide chain of the protein, the two oligosaccharide prosthetic groups being present in CN-2 (molecular weight, 15,000) and CN-3 (molecular weight, 10,000) (*5*). The complete sequences of four fragments have been published: CN-6 (the amino terminal 26 residues of the protein), CN-7, CN-8, and CN-9 (*10, 11*). Partial sequences for CN-3, CN-4, and CN-5 have also been reported (*5*).

We report here the sequence of a region in CN-1, the fragment having a molecular weight of 25,000 from the carboxy terminus of the molecule (*5*) and the structure of a peptide which provides evidence for the ordering of CN-7, CN-9, and CN-4 in the polypeptide chain. The two extensive regions of sequence thus assembled provide convincing evidence for internal homology in the polypeptide chain of transferrin.

The aminoethyl (AE) and carboxamidomethyl (CAM) derivatives of CNBr fragments were prepared and isolated as

(1) Leu-Lys-Val-Pro-Pro-Arg-Metsulphone-Asx

CN-7 CN-9 CN-4

(2) Leu-Lys-Val-Pro-Pro-Arg-Metsulphone-Asx-Ala-Lys-Metsulphone-Tyr

Fig. 1. Sequences of peptides denoting the ordering of CN-7, CN-9, and CN-4. Abbreviations: Leu, leucine; Lys, lysine; Val, valine; Pro, proline; Arg, arginine; Asx, aspartic acid or asparagine; Ala, alanine; and Tyr, tyrosine.

```
                1                                   10                                  20
Line A  LEU-PHE-Ser-SER-Pro-His-Gly-LYS-Asn-LEU-LEU-PHE-Lys-ASP-Ser-Ala-His-Gly-Phe-Leu-

Line B  LEU-PHE-Arg-SER-Glu-Thr-▬-LYS-Asp-LEU-LEU-PHE-Arg-ASP-Asp-Thr-Val-Cys-Leu-Ala-

                                   30                                  40
Line A  LYS-Val-Pro-Pro-ARG-Met-Asn-Ala-Lys-Met-TYR-LEU-GLY-Tyr-GLU-TYR-VAL-Thr-ALA-Ile-

Line B  LYS-Leu-His-Asp-ARG-Asn-Thr-Tyr-Glu-Lys-TYR-LEU-GLY-Glu-GLU-TYR-VAL-Lys-ALA-Val-

                                   50                                  60
Line A  Arg-ASN-LEU-ARG-Glu-▬▬▬▬▬-Gly-Thr-Cys-Pro-GLU-ALA-Pro-THR-Asn-Glu-Cys-Lys-

Line B  Gly-ASN-LEU-ARG-Lys-Cys-Ser-Thr-Ser-Ser-Leu-Leu-GLU-ALA-Cys-THR-Phe-Arg-
```

Fig. 2. A comparison of the amino acid sequences of two regions from the polypeptide chain of human serum transferrin. Line A consists of the combined sequences of CN-7, CN-9, and CN-4; line B consists of a region from CN-1. The amino terminal region of CN-7 and the carboxy terminal region of CN-4 are not included. Identical residues in corresponding positions are denoted with capital letters, and chemically similar residues (conservative replacements) are underlined. The numbering refers to the residues within the alignment, and not to positions in the polypeptide chain of transferrin.