classes of heavy chains differ in length and are not uniform in homology from domain to domain, phylogenetic trees cannot be based on a single small segment such as the hinge region or the COOH-terminus (*19*). The individual domains and not the whole chains should be compared. The nonuniformity in homology of corresponding C region domains of human μ, α, γ, and ϵ chains suggests that the four classes evolved by recombination of primordial tandem genes coding for single domains.

TERESA L. K. LOW
YU-SHENG VICTOR LIU
FRANK W. PUTNAM

Department of Zoology, Indiana University, Bloomington 47401

References and Notes

1. For the nomenclature of immunoglobulins and schematic diagrams of their polypeptide chain structure and of the Fab and Fc pieces of immunoglobulins A, M, and G (IgA, IgM, and IgG), see F. W. Putnam (*2*). Abbreviations of the amino acid residues are as follows: Lys, lysine; His, histidine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Asx, aspartic acid or asparagine, identity not established; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Glx, glutamic acid or glutamine, identity not established; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Ile, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Trp, tryptophan.
2. F. W. Putnam, in *Progress in Immunology II*, L. Brent and J. Holborow, Eds. (North-Holland, Amsterdam, 1974), vol. 1, pp. 25–37; *Science* **163**, 633 (1969); *J. Hum. Evol.* **1**, 591 (1972).
3. V. Moore and F. W. Putnam, *Biochemistry* **12**, 2361 (1973); F. W. Putnam, T. Low, V. Liu, H. Huser, E. Raff, F. C. Wong, J. R. Clamp, *The Immunoglobulin A System*, J. Mestecky and A. R. Lawton, Eds. (Plenum, New York, 1974), pp. 177–189.
4. B. Frangione and C. Wolfenstein-Todel, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3673 (1972); E. Mendez, B. Frangione, E. C. Franklin, *Biochemistry* **12**, 5186 (1973); C. Wolfenstein-Todel, F. Prelli, B. Frangione, E. C. Franklin, *ibid.*, p. 5195.
5. C.-Y. Chuang, J. D. Capra, J. M. Kehoe, *Nature (London)* **244**, 158 (1973).
6. A. G. Plaut, R. Wistar, Jr., J. D. Capra, *J. Clin. Invest.* **54**, 1295 (1974).
7. Y. Tsuzukida and F. W. Putnam, IgA2κ, $Am_3(+)$ allotype, unpublished results; A. Toraño and F. W. Putnam, IgA2λ, $Am_3(-)$ allotype, unpublished results.
8. F. W. Putnam, G. Florent, C. Paul, T. Shinoda, A. Shimizu, *Science* **182**, 287 (1973).
9. G. M. Edelman, B. A. Cunningham, W. E. Gall, P. O. Gottlieb, U. Rutishauser, M. J. Waxdal, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 78 (1969); E. M. Press and N. M. Hogg, *Biochem. J.* **117**, 641 (1970).
10. H. Bennich and H. von Bahr-Lindström, in *Progress in Immunology II*, L. Brent and J. Holborow, Eds. (North-Holland, Amsterdam, 1974), vol. 1, pp. 49–58.
11. M. S. Halpern and M. E. Koshland, *Nature (London)* **228**, 1276 (1970); J. Mestecky, J. Zikan, W. T. Butler, *Science* **171**, 1163 (1971); J. Mestecky *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 544 (1974).
12. D. R. Davies, E. A. Padlan, D. M. Segal, *Annu. Rev. Biochem.* **44**, 639 (1975); R. J. Poljak, *Adv. Immunol.* **21**, 1 (1975); A. B. Edmundson, K. R. Ely, E. E. Abola, M. Schiffer, N. Panagiotopoulos, *Biochemistry* **14**, 3953 (1975).
13. J. Baenziger and S. Kornfeld, *J. Biol. Chem.* **249**, 7270 (1974).
14. Model prediction for IgA Fc was done by Margaret H. Garner using x-ray coordinates for Fab structures and the tables of P. Y. Chou and G. Fasman [*Biochemistry* **13**, 211 (1974)].
15. K. J. Dorrington and R. H. Painter, in *Progress in Immunology II*, L. Brent and J. Holborow, Eds. (North-Holland, Amsterdam, 1974), vol. 1, pp. 75–84.
16. D. E. Isenman, K. J. Dorrington, R. H. Painter, *J. Immunol.* **114**, 1726 (1975).
17. J. M. Kehoe, A. Bourgois, J. D. Capra, M. Fougereau, *Biochemistry* **13**, 2499 (1974); M. Colomb and R. R. Porter, *Biochem. J.* **145**, 177 (1975).
18. M. M. Hurst, J. E. Volankis, R. B. Hester, R. M. Stroud, J. C. Bennett, *J. Exp. Med.* **140** 1117 (1974).
19. H. L. Spiegelberg, *Nature (London)* **254**, 723 (1975).
20. We thank J. Madison, G. East, E. Dodge, D. Radjeski, J. Novotny, and J. Dwulet for technical assistance; Dr. Elliot Ossermann, Institute for Cancer Research, Columbia University, New York, for plasma from patient Bur; and Dr. A. G. Plaut, Tufts University School of Medicine, for IgA protease. The sequence of the λ light chain of Bur IgA was determined by A. Infante. Supported by grants CA-08497 from NIH and IM-2C from the American Cancer Society. Contribution No. 1014 of the Zoology Department, Indiana University.

26 September 1975; revised 3 November 1975

Genetic Characteristics of the HeLa Cell

Abstract. *The genotype of the patient Henrietta Lacks from whose cervical carcinoma the HeLa cell was derived was deduced from the phenotypes of her husband and children, and from studies of the HeLa cell. Hemizygous expression of glucose-6-phosphate dehydrogenase in HeLa, together with the deduced heterozygosity of Mrs. Lacks, is consistent with clonal origin of her neoplasm.*

HeLa, the first established human cell line, originated from a biopsy of a cervical carcinoma of a black patient, Henrietta Lacks. The biopsy was taken at the Johns Hopkins Hospital on 8 February 1951 and the cell line was established by G. O. Gey. In 1971 Jones *et al.* (*1*) reported that review of the histologic characteristics of the original biopsy suggests that Mrs. Lacks' cancer was an adenoepidermoid tumor, an unusual type for the cervical site. This may account for the unusual gross appearance, particularly malignant clinical course, and unusual in vitro characteristics of Mrs. Lacks' neoplasm.

Over the past quarter century, the HeLa cell has made major contributions to cell biology. Gartler (*2*) reported that 20 human heteroploid cell lines had identical glucose-6-phosphate dehydrogenase (G6PD) and phosphoglucomutase (PGM) phenotypes: G6PD(A) and PGM(1-1). Although G6PD(A) is found mainly in blacks, some of the heteroploid cell lines were known to have been derived from whites. By examining the G6PD and PGM phenotypes in a variety of transformed cell lines, Gartler found that in no case were the phenotypes of G6PD and PGM different or modified from the normal tissue, which suggests that the phenotypes found were not an artifact of establishment in culture per se. He concluded that contamination with HeLa cells was the most likely explanation for G6PD(A) and PGM(1-1) phenotypes found in all heteroploid human cell lines. The problem of possible contamination of other long-term cultured tumor cell lines (*3*) with HeLa cells not only caused an international embarrassment, but also raised the concern of misattributing a specific property to another cell line, for example, a virus or a tumor-specific marker, which actually belongs to HeLa.

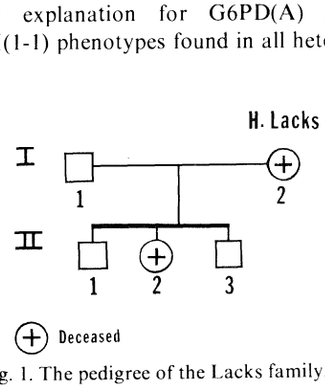

Fig. 1. The pedigree of the Lacks family.

With the continued and growing use of tissue culture in biochemical research, intra- and interspecific contamination becomes a significant risk. The determination of stable genetic markers on cultured cells is a powerful tool for monitoring such contamination. Recent experiments in which cultured cells and innumerable clones of somatic cell hybrids have been used for genetic analysis have shown that, with the proper use of polymorphic markers to characterize the cells, the possibility of undetected cross contamination of cultures is no longer the problem it once may have been (*4*). Therefore, in an effort to clarify the characteristics of the HeLa cell and establish its probable genotype for better-known polymorphisms, we studied HLA and other markers in the surviving husband and children of Henrietta Lacks.

The pedigree of the Lacks family is given in Fig. 1. The genetic markers are shown in Table 1. Henrietta Lacks' ABO and Rh types were known from her medical records—type O, Rh positive. Most of her other red cell antigens were deduced from the genotyping of her children and from data published by Kelus *et al.* (*5*), who used the method of mixed agglutination to demonstrate the M, N, S, s, and Tj^a antigens on HeLa cells.

In Table 1, the genetic markers indicated by a section symbol (§) could not be deduced for Mrs. Lacks with certainty. These included the red cell antigens systems, Jk^a, Fy^a, Fy^b, P_1, Le^a, Le^b, and Se, and the serum protein, haptoglobin. There is no doubt that she possessed Gm(1,5), but whether she also possessed GM(3,4),

GM(6), or InV(1) cannot be determined. On electrophoresis of sonicated cultured HeLa cells, we found the following red cell enzymes phenotypes: AK(1-1) for adenylate kinase, ADA(1-1) for adenosine deaminase, PGM(1-1), 6PGD(A) for 6-phosphogluconic dehydrogenase, and G6PD(A). Since Mrs. Lacks had both G6PD(A) and G6PD(B) sons, she must have been heterozygous (that is, AB). Bengtsson *et al.* (*6*) previously reported that HeLa cells express the acid phosphatase (AcP) phenotype AcP(AB).

The most useful genetic marker for HeLa cells appears to be the HLA antigens. From the family study, Mrs. Lacks' genotype could be either *A28,-/A2,BW40* or *A28,-/A3,BW35*. The HLA typing of HeLa cells was consistent with the possession of A28,A3,BW35. Kennett *et al.* (*7*) have also reported that, on the basis of HLA typing of HeLa cells and intraspecific somatic cell hybrids made with HeLa cells, the HLA antigens expressed on HeLa are A28,A3,BW35. Thus the HeLa genotype is probably *A28,-/A3,BW35*. The probability of possessing this combination of HLA haplotypes is 2.95×10^{-4} in blacks and 1.01×10^{-4} in whites, while the frequencies of the corresponding HLA phenotypes are, respectively, 16.43×10^{-4} and 1.54×10^{-4}.

The HLA antigens are known to be present on all nucleated cells and are stable in vitro, as evidenced by their presence on HeLa cells even after many years in culture (*7, 8*). If the HLA antigens of the donors of the various tumor cell lines can be determined, one can monitor for contamination by determining the HLA antigen expression. Even if it is impossible to obtain the HLA type on a donor's lymphocytes, the probability of another cell line with the exact array of HLA and G6PD types [HLA-A28,A3; BW35; and G6PD(A)] is about 3.3×10^{-4} in blacks. It is difficult to calculate a corresponding frequency for whites because of the differences in G6PD frequencies between some Mediterranean and most other Caucasoid populations. With the exception of the Mediterranean area, the G6PD(A) frequency is essentially zero and this is enough to characterize the difference between HeLa and Caucasoid-derived lines, as pointed out by Gartler (*2*).

On the basis of the Lyon hypothesis and studies of the X-linked marker G6PD, conclusions on the unicellular versus multicellular origins of neoplasms have been made. For example, in females heterozygous for the AB electrophoresis polymorphism of G6PD, uterine leiomyomas (*9*), the red cells in chronic myeloid leukemia (*10*), and metastatic nodules of colon cancer (*11*) were either type A or B, suggesting unicellular origin, whereas trichoepitheliomas (*12*) and carcinoma of the liver and breast (*13*) were type AB, suggesting multicellular origin.

The findings that Mrs. Lacks must have been heterozygous, G6PD(AB), and that all HeLa lines tested by others (*2, 14*) as well as by us are G6PD(A) suggest a unicellular origin of her cervical carcinoma. Other possibilities include that, although she was genotypically heterozygous, (i) Mrs. Lacks was phenotypically G6PD(A) because of the chances of X inactivation, (ii) Mrs. Lacks' tumor originated from multiple cells that by reason of being in a patch of clonal origin or merely by chance were all G6PD(A), (iii) only a single cell (or descendants of a single cell) in a tumor of multicellular origin survived in culture and gave rise to the established cell line HeLa, or (iv) that one X chromosome (and the same X chromosome) has been lost from HeLa cells. Relevant to the second possibility that selection for G6PD(A) occurred is the observation (*9*) of a culture from a G6PD heterozygote in which the ratio of A to B changed from 40 : 60 to 80 : 20 over 25 passages in culture. Relevant to the fourth possibility are the obser-

Table 1. The genetic markers of the Lacks family. Abbreviations of the genetic markers tested are as follows. Included in the red cell antigen system are: A, A_1, B, O; Rh (D, C, c, E, e, V); M, N, S, s; Kell (K, k, $Kp^{a,b}$, $Js^{a,b}$); Kidd ($Jk^{a,b}$); Lutheran ($Lu^{a,b}$); Lewis ($Le^{a,b}$); Duffy ($Fy^{a,b}$); P (P_1, Tj^a); Xg (Xg^a); and secretor status (Se). Included among the genetic markers in plasma are the group-specific component (Gc), haptoglobin (Hp), transferrin (Tf), the immunoglobulin heavy chain marker (Gm system), and the light chain marker (InV system). The red cell enzyme markers are explained in the text.

Genetic markers	Husband 1-2	Child II-1	Child II-2	H. Lacks*	HeLa
A	+†	+	+	0	
A_1	0‡	0	0	0	
B	0	0	0	0	
D	+	+	+	+	
C	0	0	+	+	
c	+	+	+	+	
E	0	0	0	0	
e	+	+	+	+	
V	+	+	+	+	
M	+	0	0	+	+
N	+	+	+	+	+
S	+	+	+	+	+
s	+	+	+	+	+
K	0	0	0	0	
k	+	+	+	+	
Kp^a	0	0	0	0	
Kp^b	+	+	+	+	
Js^a	0	0	0	0	
Js^b	+	+	+	+	
Jk^a	+	+	+	+	
Jk^b	+	+	+	0/+§	
Lu^a	0	0	0	0	
Lu^b	+	+	+	+	
Fy^a	0	0	0	0/+§	
Fy^b	+	+	+	0/+§	
Xg^a	+	+	0	+	
P_1	+	0	0	0/+§	
Tj^a	+	+	+	+	+
Le^a	+	+	+	0/+§	
Le^b	0	0	0	0/+§	
Se	0	0	0	0/+§	
ADA	1-1	1-1	1-1	1-1	1-1
AK	2-1	2-1	1-1	1-1	1-1
AcP	BB	AB	AB	AB	AB
PGM	2-1	1-1	2-1	1-1	1-1
6PGD	AA	AA	AA	AA	AA
G6PD	B	B	A	AB	A
Gc	1-1	1-1	1-1	1-1	
Hp	1-1	1-1	1-1	1-1/2-1§	
Tf	c-c	c-c	c-c	c-c	
Gm	(1.5)(3.4)	(1.5)	(1.5)	(1.5)(1.5 or +/0§ 3.4 or 6)§	
InV(1)	0	0	0	(0/+§)	
HLA	*A3,BW35/ A2,BW40*	*A3,BW35/ A2,BW40*	*A3,BW35/ A28,-*	*A3,BW35/ A28,-*	A3,A28,BW35

*Deduced from family data or direct study of HeLa. †Positive. ‡Negative. §Cannot be deduced with certainty.

vations (*15*) that several strains of HeLa or presumed HeLa have at least two X chromosomes as identified by banding techniques. These may of course be replicas of one chromosome.

SUSAN H. HSU, BERNICE Z. SCHACTER
NANCY L. DELANEY, THOMAS B. MILLER
VICTOR A. MCKUSICK
Division of Medical Genetics, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
R. H. KENNETT, J. G. BODMER
D. YOUNG, W. F. BODMER
Genetics Laboratory, Department of Biochemistry, University of Oxford, Oxford, England

References and Notes

1. H. W. Jones, V. A. McKusick, P. S. Harper, K. D. Wuu, *Obstet. Gynecol.* **38**, 945 (1971).
2. S. M. Gartler, *Nature (London)* **217**, 750 (1968).
3. B. J. Culliton, *Science* **184**, 1058 (1974).
4. *New Haven Conference* (1973): *First International Workshop on Human Gene Mapping*. Birth Defects: Original Article Series, X, Number 3, 1974.
5. A. Kelus, B. W. Gurner, R. R. A. Coombs, *Immunology* **2**, 262 (1959).
6. B. O. Bengtsson, M. Nabholz, R. Kennett, W. F. Bodmer, S. Povey, D. Swallow, *Somatic Cell Genet.* **1**, 41 (1975).
7. R. H. Kennett, B. Hampshire, B. Bengtsson, W. F. Bodmer, *Tissue Antigens* **6**, 80 (1975).
8. V. C. Miggiano, M. Nabholz, W. F. Bodmer, in *Histocompatibility Testing 1970* (Munksgaard, Copenhagen, 1970), pp. 623–629.
9. D. Linden and S. M. Gartler, *Science* **150**, 69 (1965).
10. P. J. Fialkow, S. M. Gartler, A. Yoshida, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1468 (1967).
11. E. Beutler, Z. Collins, L. E. Irwin, *N. Engl. J. Med.* **276**, 389 (1967).
12. S. M. Gartler, L. Ziprkowski, A. Krakowski, R. Ezra, A. Szeinberg, A. Adam, *Am. J. Hum. Genet.* **18**, 282 (1966).
13. P. R. McCurdy, *Clin. Res.* **15**, 65 (1967).
14. S. M. Gartler, *Natl. Cancer Inst. Monogr.* **26**, 167 (1967); M. W. Steele, *Biochem. Genet.* **4**, 25 (1970).
15. W. A. Nelson-Rees, R. R. Flandermeyer, P. K. Hawthorne, *Science* **184**, 1093 (1974); W. A. Nelson-Rees, V. M. Zhdanov, P. K. Hawthorne, R. R. Flandermeyer, *J. Natl. Cancer Inst.* **53**, 751 (1974); O. J. Miller, D. A. Miller, P. W. Allderdice, V. G. Dev, M. S. Grewel, *Cytogenetics* **10**, 338 (1971); U. Francke, D. S. Hammond, J. A. Schneider, *Chromosoma* **41**, 111 (1973).
16. Supported in part by NIH grant GM-19489, and also by a grant from the United Kingdom Medical Research Council. We thank J. R. Hutchinson for technical assistance and R. Sanger for confirmation of some red blood cell typings.

3 September 1975; revised 17 October 1975

Visual Adaptation: Effects of Externally Applied Retinal on the Light-Adapted, Isolated Skate Retina

Abstract. *Incubation with externally applied 11-*cis *retinal induces a marked increase of visual sensitivity within partially bleached skate photoreceptors. This activity of 11-*cis *retinal is duplicated by 9-*cis *retinal, but not by all-*trans *retinal. The sensitization of photoreceptors promoted by 11-*cis *and 9-*cis *retinal is accompanied by the formation of rhodopsin and isorhodopsin, respectively.*

All visual pigments consist of retinal (vitamin A aldehyde) attached to a protein, opsin. The configuration of the retinal chromophore appears crucial for the function of these pigments, since visual excitation is believed to involve the isomerization of the chromophore from the 11-*cis* to the all-*trans* form (*1*). Rhodopsin, the most extensively studied visual pigment, consists of 11-*cis* retinal joined to the opsin found in vertebrate rods. When extracted into digitonin, rod opsin can also combine with 9-*cis* retinal to form isorhodopsin (*2*); however, substantial evidence indicates that isorhodopsin is virtually nonexistent in vivo under normal conditions (*1, 3*).

In both vertebrates and invertebrates, the role of the visual pigments in mediating visual excitation is well established. For example, the excitation spectra of visual systems agree quite well with the absorption spectra of the visual pigments present in the photoreceptors (*4*). In the vertebrate rod, the content of rhodopsin also appears to be closely related to the logarithm of visual sensitivity during dark adaptation. Three types of evidence support this view: (i) after strong light adaptation, the recovery of log sensitivity during the "slow," or photochemical, phase of dark adaptation proceeds in parallel with the regeneration of rhodopsin (*5–7*); (ii) if the regeneration of rhodopsin after strong light adaptation is prevented by isolation of the retina from the back of the eye, the retina does not recover its original, dark-adapted level of sensitivity (*8*); and (iii) rats deficient in vitamin A exhibit both a depressed level of rhodopsin and substantially decreased visual sensitivity (*9*). As yet, however, no one has induced the formation of rhodopsin in an isolated retina and observed an increase in visual sensitivity.

We report here that 11-*cis* retinal, externally applied to the light-adapted, isolated all-rod retina of the skate (*6, 7, 10*), promotes both the rapid formation of rhodopsin and a sharp increase in the sensitivity of the photoreceptors. In addition, we present evidence that externally applied 9-*cis* retinal leads to increased receptor sensitivity with the formation of isorhodopsin in the photoreceptors.

Eyes from dark-adapted skates (*Raja oscellata* or *R. erinacea*) were enucleated, hemisected, and drained of vitreous humor under dim red light. Rectangular pieces of eyecup measuring approximately 3 mm by 5 mm were trimmed from the tapetal region of the eye and soaked in a skate Ringer solution containing 75 m*M* sodium L-aspartate (*11*). The L-aspartate suppresses the activity of neurons in the retina proximal to the photoreceptors, thereby isolating the receptor response (*12*). For electrophysiological recording, the retina was isolated from the eyecup, mounted upon moistened filter paper with the photoreceptor layer up, and positioned in a shielded cage under a gentle stream of moist oxygen. All adapting exposures (bleaches) and test flashes were carried out with light spectrally shaped by passage through a Kodak Wratten 58 (green) filter and through Schott-Jena KG-1 and KG-3 heat filters. The light was attenuated by neutral density filters and focused to give full-field illumination of the retina from above. The photoreceptor potential was recorded extracellularly across the retina (*7*). The recording electrode, placed at the surface of the receptor layer, was a glass pipette containing a wick of glass fibers and filled with aspartate Ringer solution; the reference electrode, a grounded loop of chlorided silver wire, was positioned beneath the preparation. Responses elicited by 0.2-second test flashes were amplified (bandpass of 0.1 to 1000 hertz), displayed on an oscilloscope, and recorded on a pen oscillograph. A photoreceptor response of amplitude 3 μv was used as a criterion to determine threshold intensities of the test flash; photoreceptor sensitivity was defined as the reciprocal of the measured threshold. Spectrophotometry was performed with an apparatus previously described (*13*); for measurements of transmissivity before and after the application of retinal, the isolated retina was mounted (with the photoreceptor layer up) on a piece of Vitallium mesh (Howmet Corp.), which lay in contact with moistened filter paper in a clear plastic dish.

Figure 1 describes the results of two typical electrophysiological experiments. In the first experiment (upper curve), we exposed a dark-adapted isolated retina to intense illumination which bleached more than 90 percent of the rhodopsin initially present (*14*). Threshold measurements made after the offset of the adapting light showed that the sensitivity of the photoreceptors had been reduced by the adapting illumination to a stable value, approximately 4 log units less than the dark-adapted value (*15*). Aliquots of all-*trans* retinal or 11-*cis* retinal suspended in ethanol-aspartate Ringer solution were then applied dropwise to the upper surface of the retina at the times indicated by the arrows in Fig. 1. Repeated threshold determinations showed that the sensitivity of the photoreceptors was unaffected by the