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THE INHERITANCE OF ACQUIRED CHARACTERISTICS*

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KEY WORDS: inheritance of acquired characteristics, Lamarckism, epinucleic inheritance, extranucleic inheritance, Lamarckian inheritance, saltatory evolution, punctuated equilibrium, Lysenkoism, nonmendelian inheritance

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INTRODUCTION

Can environmentally induced or acquired changes in organisms be transmitted to future generations? Does the inheritance of acquired characteristics (IAC)—if it occurs at all—play a significant role in evolution? These questions were the subject of passionate debate and heated political controversy in the late 19th century and in the first six decades of the 20th (11, 30, 56, 71).

*Dedicated to the Memory of Tracy M. Sonneborn, Pioneer

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The decisive successes of classical genetics and molecular biology submerged this debate, and the take-home lesson for most biologists and geneticists has been that there is no such thing as inheritance of acquired characteristics. Thus, among 30 of the most widely used college textbooks of genetics published since 1962, none indicated that actual examples of IAC had been found and only seven even mentioned IAC or Lamarck. The following statements are typical in their dismissal of the concept of IAC:

> Inheritance of acquired characteristics' : The idea *(apparently false)* that features developed during the life of an organism can be passed on to offspring by altered heredity (53).

> Lamarck's hypothesis of the inheritance of acquired modifications has been *discarded* because *no molecular mechanism exists* or *can be imagined* that would make such inheritance possible (76).

This paper reconsiders the subject of IAC in light of our present, much-improved understanding of the molecular mechanisms of both long-known and newly described IAC systems. Five fundamentally different mechanisms are distinguished that all give rise to IAC. Essential experimental details of eight prototype systems are described and their underlying mechanisms outlined.

It is shown that the observations concerning IAC are fully compatible with current concepts of molecular genetics and that IAC and Mendelian inheritance coexist comfortably in the universe of molecular biology. In this new context, a fresh perspective on inheritance and evolution is presented.

DEFINITION OF INHERITANCE OF ACQUIRED CHARACTERISTICS (IAC); CLASSIFICATION OF IAC SYSTEMS

The chief features of ten IAC systems are shown in Table 1. The relationship of these systems to each other and to classical Mendelian inheritance is depicted in Figure 1.

The systems described in lines 1–8 of Table 1 are defined operationally as IAC systems because they conform to the following experimental pattern: Individual organisms or cultures of cells incubating in a particular environment are exposed briefly to a chemical or physical treatment under conditions that allow little or no growth (thereby ruling out selection of mutants). *Following the exposure, and upon being returned to the original environment, all or a large proportion of the treated cells (or organisms) exhibit new characteristics that are passed on heritably to succeeding generations.* This phenomenology is completely contrary to the behavior expected of Mendelian traits but it accords well with behavior anticipated of acquired traits.

The word "acquire" is used in this paper in conformance with two of the

definitions of Webster's Dictionary: (*a*) "to come into possession of" and (*b*) "to come to have as a characteristic". A more active mode of acquisition described by a third definition, (*c*) "gained as a result of effort or experience" is not exemplified by any of the systems under review (e.g. the giraffe's long neck; the blacksmith's strong arm). Historically, discussions of IAC have often ignored this distinction.

Despite the similarities in the experimental procedures that trigger the changes in heritable characteristics shown in lines 1–8 of Table 1, four sharply different mechanisms of heritability are responsible: (*a*) heritably stabilized gene expression (lines 1, 2, 3); (*b*) cortical inheritance, i.e. clonally transmitted physical alterations in morphology (line 4); (*c*) DNA modification, e.g. clonally transmitted changes in DNA methylation (lines 5, 6); (*d*) induced loss of specific nonessential nucleic acid elements (lines 7, 8). A fifth mechanism—acquisition of foreign nucleic acid sequences—(lines 9, 10) is discussed later.

EXPERIMENTAL SYSTEMS THAT DEMONSTRATE IAC

Systems Based on Heritably Stabilized Gene Expression

TRANSFORMATION OF SEROTYPES IN *PARAMECIUM AURELIA* The cilia and surface of each paramecium display characteristic proteins, 250–310 kd in size, which determine their serotype (20). The serotypes are identified by means of specific antisera: Two hours of incubation in dilute homologous antiserum immobilize the paramecia. Immobilization is followed by transformation to a new serotype (5). A particular stock (strain) of *Paramecium* has the potential to express about 12 different serotypes. These are determined by genes at 12 different loci, but, except during transitional states, each homozygous animal normally displays only one serotype at a time ["Mutual exclusion" (16, 19)]. The expression of a serotype is clonally transmitted, i.e. once transient incubation in antiserum effects a change in serotype, the new serotype is heritably and stably transmitted to the progeny. Changes in serotype can be induced not only by antisera but also by a series of other environmental agencies, namely, changes in temperature, pH, abundance of food supply or salinity, and treatment with trypsin or chymotrypsin and other substances (5). The initial serotype of the treated paramecia also plays an important role in channeling the induced serotype changes.

The changes are reversible since animals from a particular stock can be induced to go back to their "original" serotype by controlled changes in the environment. The flexibility of this system is quite impressive, especially if one recalls that each serotype locus is represented by about 1000 copies in the macronucleus of each animal (19).

In a representative experiment, paramecia of variety 1, serotype 41G, growing at 24°C were exposed to 36°C for 2 1/2 hr and then moved back to 24°C (one fission takes about 4 hr). The heat-treated paramecia showed no outward change immediately after treatment but some hours later 50% had changed from serotype 41G to 41D. With longer treatments 100% could be transformed (6). In the absence of heat treatments, serotypes 41G and 41D each reproduced at 24°C without change for a long period of time. A plausible interpretation of the observations is as follows: A controlling mechanism or substance heritably suppresses expression of all but one of the serotype-determining genetic loci. [Only one serotype-determining mRNA was detected (12, 64)]. The suppression pattern can be destabilized by a great variety of environmental treatments (5). These shift suppression to a new set of serotype genes, leaving one gene active (19) (Table 1, line 1 and footnote *a*).

INHERITANCE OF THE WALL-LESS CONDITION IN BACTERIA (*BACILLUS SUBTILIS* MASS-CONVERSION STABLE L FORMS) (40, 41, 43) In most bacterial species it is a fairly routine procedure to remove the cell wall. Removal may be achieved by using the enzyme lysozyme that depolymerizes peptidoglycan (the principal rigid constituent of most bacterial walls), or by inhibiting peptidoglycan synthesis in growing cultures with penicillin or other inhibitors. Once peptidoglycan has been removed, the other wall constituents are usually lost, leaving only protoplasts—cells completely devoid of cell wall. In our model system, *Bacillus subtilis*, each rod-shaped bacillus gives rise to 1–3 protoplasts after 20–30 minutes of lysozyme treatment. Media of high solute content must be used to prevent lysis of the protoplasts. Even when they are suspended in hypertonic media, protoplasts only increase in size but are unable to divide or to replace the previously removed cell wall. In liquid media, the presence of the cell wall is evidently required for cell division to take place or new cell wall to form.

The situation changes in a most surprising way when the protoplasts are transferred to soft-agar media. In this medium, each protoplast can give rise to an L colony—a slow-growing colony consisting of spherical, membrane-bounded "L bodies" of very heterogeneous size. The soft agar evidently allows the burgeoning protoplasts to be subdivided into viable fragments. The fragments, L bodies, in turn are capable of indefinite further propagation: Upon transfer to fresh soft-agar media, they give rise to new L colonies. By contrast, if the L bodies or protoplasts are plated on hard agar or gelatin media, prompt reversion to the walled, rod-shaped state occurs and only normal bacterial colonies are produced (40, 43).

Experiments have shown that the sharp difference in heritable persistence of protoplasts and L bodies on soft agar on the one hand and on hard agar or gelatin on the other is due to a changed equilibrium between peptidoglycan

Table 1 Descriptive outlines of prototype systems showing inheritance of acquired characteristics

Line #	Mechanism				Process of trait acquisition					References
	# Type	Passes germ line	Description	System	Heritable state #1	Inducing treatment → ---------- ← Reversing conditions	Heritable state #2	Efficiency of conversion	Changing trait	
1	1 Extra-nucleic	n.a.[1]	Switch in stable blockage of all-but-one of the serotype genes[a]	Eukaryote Single cell	Paramecium of serotype 41G replicating at 24°C	36°C for 5 hr → induces loss of 41G antigen, gain of 41D ---------- ← temp. manipulat.	Paramecium of serotype 41D replicating at 24°C	→ 100% ---------- 100% ←	Serotype 41G ⇵ 41D	6, 19
2	1 Extra-nucleic	n.a.	Change in equilibrium between post-translational gene products	Prokaryote Single cell	*Bacillus subtilis* bacteria propagating in soft agar	30 min in Lysozyme removes wall. → ---------- ← 60 min in gelatin: L forms revert	L-forms (protoplast-derived) propagating in soft agar	→ 100% ---------- 100% ←	± cell wall ± ability to divide in liquid	40, 43
3	1 Extra-nucleic	n.a.	Transcription switch maintained in "on" position	Prokaryote Single cell	*E.coli* w/Lac operon uninduced replicating in maintenance conc. of inducer, 5×10^{-6} M TMG	3 doublings in → inducing conc. of inducer 5×10^{-4} M TMG ← replication in absence of TMG	*E.coli* w/Lac operon induced replicating in maintenance conc. of inducer. 5×10^{-6} M TMG	→ 100% ---------- 100% ←	± β-galactosidase and permease	60, 63
4	2 Extra-nucleic	n.a.	Cortical inheritance[b]	Eukaryote Single cell	*Oxytricha fallax* singlets (normal animals) reproducing by normal fission	2 singlets fuse → to form doublet ← longitudinal cut cleaves doublet into 2 singlets	*Oxytricha fallax* doublets reproduce by fission, producing more doublets	N.A. ---------- 100% ←	2 singlets ⇵ 1 doublet	3, 25, 26

Table 1 (*Continued*)

Line #	Mechanism: # Type	Passes germ line	Description	System	Process of trait acquisition: Heritable state #1	Inducing treatment → / ← Reversing conditions	Heritable state #2	Efficiency of conversion	Changing trait	References
5	3 Epi-nucleic	n.a.	DNA demethylation converts uncommitted cells to determined ones [c]	Eukaryote tissue culture line	Undifferentiated mouse fibroblasts dividing	3μM 5-azacytidine triggers → demethylations / ← not reversible	Stem cell line producing myocytes	→ 25% of clones produce myocytes not rev. ←	uncommitted cells ⇵ determined cells	14, 39
6	3 Epi-nucleic	n.a.	Restriction-modification mediated by glucosylation of DNA [d]	Phage	Phage T2$_+$ with glucosylated DNA replicating in *E.coli* strain B	Glucosylation lost → growing in B rgl/4$_0$ / ← Glucosylation regained growing in Shigella	Phage T2 with unglucosylated DNA replicating in *E.coli* B rgl/4$_0$	→ 100% / 100% ←	± DNA glucosylation	21, 51, 68
7	4 Nucleic	n.a.	Streptomycin induces loss of chloroplasts and chloroplast DNA [e]	Eukaryote Single cell	Euglena with functional chloroplasts replicating in 0.15% butyrate medium in the light	Incubation for → 6 days in 160 μg/ml streptomycin medium / ← not reversible	Euglena devoid of chloroplasts replicating in 0.15% butyrate medium in the light	→ 100% / not ← reversible	± chloroplasts	66, 67

8	4 Nucleic	Yes	Heat cures infection by SS RNA rhabdovirus[e]	Eukaryote Insect	Drosophila sensitive to CO_2, carrying virus Sigma, reproducing	Hold flies above → 30°C for six days during gametogenesis --- ← inject flies with virus	Drosophila resistant to CO_2, virus-free, reproducing	→ 100% --- n.a. ←	± CO_2 sensitivity ± Sigma virus	10, 48, 65
9	5 Nucleic	n.a.	Acquisition of multiple antibiotic resistance plasmid (DNA)	Prokaryote Single cell	rif^r *E.coli* strain replicating[f]	Conjugal transfer → of plasmid R26 from "any" gram- donor to rif^r *E.coli* --- ← cure plasmid	rif^r *E.coli* carrying → R26 plasmid, replicating	.1-.001% --- 100% ←	± plasmid ± resistances ± conjug. factor	27, 72
10	5 Nucleic	Yes	Acquisition of retrovirus; becomes endogenous provirus SS RNA → DS DNA	Eukaryote Mouse	"Brown" mice free from infection by Gross murine leukemia virus, reproducing	Infection by murine leukemia virus → --- ← Excision of virus from chromosome #9	"Dilute brown" mice carrying Gross murine leukemia virus on chromosome #9, reproducing[g]	→ Rare --- 3.3×10^{-5} ←	± virus	32

[a] In *trypanosomes*, also, all but one of many surface antigen genes is suppressed, but switch-over to new antigen is due to mutation. The mechanism of mutual exclusion is unknown in either system (16).

[b] Two metazoan systems reminiscent of cortical inheritance are sketched below:

1. In **transdetermination** of imaginal discs of *Drosophila*, the discs—developmentally determined cell aggregates—can switch from one fate to another, e.g. from leg precursor to antenna precursor. Several **adjacent** cells switch simultaneously and then **each** transmit the switched potential **clonally** (polyclones) (79).

2. Flatworms of the genus *Stenostomum incaudatum* were grown in dilute lead acetate for four generations. A **single** individual, "C", then produced the following offspring: **an inviable animal **two normals **a double monster (M1): two ventrally joined animals. This M1, observed for ten generations, gave rise to 188 double monsters, **another double monster (M2): two dorsally joined animals. M2, observed for eight generations produced 94 similar offspring (73).

[c] Table 1 of Holliday's review (29) cites 18 references to 5-azacytidine-promoted activation of eight different mammalian enzymes. 5-azacytidine also activates integrated retroviruses and inactive x-chromosomes of females.

[d] The environments sustaining heritable states #1 and #2 are not identical: the genotypes of the bacterial hosts differ.

[e] Other nucleic acid elements subject to curing: I. Mitochondria of yeast (cured by: ethidium bromide, acridine dyes, heat, etc) (23, 62). II. The male-progeny killing "sex ratio" spiroplasma of *Drosophila* (cured by heat). (81). III. The bacterium-like agent Kappa of Paramecium—produces toxin paramecin and "killer" phenotype—(cured by: heat, x-rays, chloramphenicol, etc.) (33). IV. The double stranded RNA reovirus of yeast—produces toxin and "killer" phenotype—(cured by: heat, cycloheximide, 5-fluorouracil) (8). V. Nonlysogenic strains of *E. coli* can be isolated from Lambda-lysogenic ones (agents: UV, mitomycin) (45). VI. Growth in ethidium bromide generally eliminates plasmids from bacterial hosts (e.g. F., R., col.) (9); proviruses can often be dislodged from their sites in animal chromosomes by iodo-or-bromodeoxyuridine (80).

[f] The rif^r trait is used in counter-selection against multiply resistant donor strains.

[g] "Dilute brown" is due to inactivation of the brown locus by insertion of the leukemia virus (32).

[h] n.a.: not applicable

biosynthesis and peptidoglycan destruction by autolysins in the two media. (Autolysins are wall-depolymerizing enzymes required by bacteria to loosen the rigid peptidoglycan envelope as growing bacilli expand and, perhaps, to aid in separating the rods in the final step of division). Protoplasts are continuously synthesizing peptidoglycan chains and continuously excreting autolysin (37, 42, 69; S. Fox, 0. E. Landman, unpublished observations). In soft-agar and liquid medium the continuous destruction of nascent peptidoglycan chains prevents accumulation of a priming quantity of cell wall; in gelatin medium (or in the presence of trypsin or other proteases) autolysin activity is inhibited (destroyed) and new cell wall can accumulate (15, 41). In different bacterial species the equilibrium between the walled and naked states is much less delicately balanced than in *B. subtilis*. Thus, mass-conversion stable L forms of *Salmonella* almost never revert to the walled state, whereas protoplasts of *B. megaterium* can scarcely be prevented from reinitiating synthesis of new cell wall (40; Table 1, line 2).

HERITABLE MAINTENANCE OF THE INDUCED STATE FOR β GALACTOSIDASE BIOSYNTHESIS IN *E. COLI* This experimental model, first described by Monod (60) and expanded by Novick & Weiner (63), illustrates how a slight modification of the ambient medium plus a brief exposure to inducing conditions can lead to a "permanent", "heritable" state of induction of the lac operon of *E. coli*.

A culture of strain B of *E. coli* growing at 37°C in a synthetic succinate medium with a 5×10^{-6} M "maintenance" concentration of the inducer thiomethyl-β-D-galactoside (TMG) is divided into subcultures A and B. Subculture A is left undisturbed. To subculture B, an "inducing" concentration of 5×10^{-4} M TMG is added and the culture is incubated until it is fully induced. The cells of the B subculture are now transferred again to medium with the 5×10^{-6} M maintenance concentration of TMG and allowed to grow indefinitely (e.g. for 180 cell generations).

Periodically both A and B are monitored for β-galactosidase activity. Culture A will not show appreciable β-galactosidase activity at any time—it was never induced. However, following the incubation in 5×10^{-4}M TMG, culture B will be permanently induced (acting like a constitutive [lac^{cst}] mutant).

In a modified, simple experimental protocol, individual induced and uninduced cells were inoculated into 5×10^{-6} M TMG medium and grown to full density. The induced cells all gave rise to fully induced cultures; the cultures grown from uninduced cells were all uninduced.

The explanation of these observations is as follows: During incubation of culture B in 5×10^{-4} M TMG inducing medium, high levels of β-galactosidase as well as β-galactoside permease were induced. Later, during growth in 5 x

10^{-6}M TMG ("maintenance") medium, the cellular permease concentrated the dilute extracellular TMG to a much higher intracellular level (e.g. 100-fold higher), thus maintaining its own induction as well as that of β-galactosidase. Subculture A, lacking high level permease, could not concentrate the dilute inducer and hence remained uninduced. (Table 1, line 3)

The three systems described above—serotype inheritance in *Paramecium*, inheritance of the wall-less state in *Bacillus subtilis*, and maintenance of the induced state in the lac operon of *E. coli*—are all "extranucleic" (46) (Figure 1): in all three there is *no change in the DNA sequences* of the cell's genome (or in DNA modification) in either nucleus or cytoplasm. Nevertheless, the molecular basis of the heritable persistence of the expressed characteristics is quite different in the three: In the serotype system, we believe it is due to the stable blockage of transcription of all but one of the serotype genes. In the *B. subtilis* system, heritable persistence in the wall-less state depends on a stabilized equilibrium between posttranslational gene products: nascent cell wall and an enzyme, autolysin, that keeps destroying wall. In the lac operon system, a transcription switch is permanently kept in the "on" position by a dilute extracellular supply of inducer boosted to a concentrated intracellular "inducing" level by permease activity acquired during an earlier induction episode (Table 1, lines 1–3).

Cortical Inheritance

Among the IAC systems reviewed in this paper cortical inheritance is the least understood in molecular terms. Cortical inheritance describes the special mode of inheritance manifested by the structures of the cell cortex and cell surface of ciliates such as *Paramecium, Tetrahymena, Stentor, Oxytricha, Stylonichia,* and *Pleurotricha* (3, 74, 75). Briefly, the experiments show that *surgical or accident-caused alterations in morphological features are propagated clonally*. The changes are inherited stably through "fissions" (cell doublings) for hundreds of generations, through repeated autogamies (self matings), and through matings with morphologically normal partners. In such mixed matings it can be arranged that the two exconjugants emerge with identical cytoplasms as well as identical genic complements yet they retain their distinctive cortical differences. The exconjugants then pass these distinctive features on indefinitely to their progeny (74). Double monsters or doublets have been among the most informative objects in cortical inheritance studies.

Doublets are formed when mating pairs of ciliates fail to separate and, instead, fuse. The doublet morphology is inherited clonally through sexual and asexual reproduction as a cortically determined trait. Doublets and singlets of *Oxytricha fallax* form cysts devoid of ciliature and other cortical features so that cysts derived from doublets and singlets are indistinguishable,