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THE INHERITANCE OF ACQUIRED CHARACTERISTICS*

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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).

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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
	Passes germ line	System	Heritable state #1	Inducing treatment → ← Reversing conditions	Heritable state #2	Efficiency of conversion	
1	1 Extra-nucleic	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	Prokaryote Single cell	<i>Bacillus subtilis</i> 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	Prokaryote Single cell	<i>E. coli</i> w/Lac operon uninduced replicating in maintenance conc. of inducer, 5×10^{-6} M TMG	3 doublings in → inducing conc. of inducer 5×10^{-6} M TMG ← replication in absence of TMG	<i>E. coli</i> 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	Eukaryote Single cell	<i>Oxytricha fallax</i> singlets (normal animals) reproducing by normal fission	2 singlets fuse → to form doublet ← longitudinal cut cleaves doublet into 2 singlets	<i>Oxytricha fallax</i> doublets reproduce by fission, producing more doublets	N.A. ----- 100% ←	2 singlets ↓ 1 doublet 3, 25, 26

Table 1 (Continued)

Line #	Mechanism		Process of trait acquisition					References		
	Passes									
	# Type	Line	Description	System	Heritable state #1	Inducing treatment → ← Reversing conditions	Heritable state #2	Efficiency of conversion	Changing trait	
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 <i>E.coli</i> strain B	Glucosylation lost → growing in B rg1/4, ← Glucosylation regained growing in Shigella	Phage T2 with unglucosylated DNA replicating in <i>E.coli</i> B rg1/4 _o	→ 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 <i>E. coli</i> strain replicating ^f	Conjugal transfer → of plasmid R26 from "any" gram-donor to rif ^r <i>E. coli</i>	rif ^r <i>E. coli</i> carrying R26 plasmid, replicating	→ .1-.001% ----- 100% ←	± plasmid ± resistance ± 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 ⁻⁶ ←	± virus	32

^aIn *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).

^bTwo 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).**

^cTable 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.

^dThe environments sustaining heritable states #1 and #2 are not identical: the genotypes of the bacterial hosts differ.

^eOther 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 Parametium—produces toxin parametium 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).

^fThe 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).

^hn.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, O. 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 $5 \times$

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 cytoplasm 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,

although doublet-derived cysts are bigger on average. Regardless of size, doublet-derived cysts always excyst as doublets. This fact permits the important inference that preexisting morphological structures do not play a major role in the heritable continuity of the acquired information. Nevertheless, the space where the morphogenetic information resides can be localized: A single cut separating an excysting doublet into halves gives rise to two clones of singlets if the cut is longitudinal and to two clones of doublets if the cut is transverse (25). This result suggests that there are two oriented regions determining global pattern in the doublet—when these are separated, each gives rise to a singlet animal clone, but when fragments of both determinative regions are retained in a single severed piece a doublet is regenerated from it (Table 1, line 4).

Other experiments with doublets have shown that cortical morphology is determined by two largely autonomous systems: one determining global pattern and the other governing the assembly of small subregions of the cortex (3, 7, 25).

By a combination of heatshock and surgery, clones of doublets were obtained in *Pleurotricha lanceolata* that exhibited mirror-image symmetry: the fused partners both had the same anterior-posterior orientation, but the mirror-image partner showed *reversal of left-right orientation* of the oral apparatus and the overall ciliature arrangement. Thus, in forming a doublet, the fused cells had *acquired bilaterally symmetric morphology*. This apparently occurred without changes in a genome previously limited to expression of the asymmetric cell morphology of singlets! Detailed examination of individual subregions of the ciliature showed that these had not followed suit in the overall mirror-image determination. Instead, subregions of the ciliature in the mirror-image half demonstrated *anterior-posterior reversed polarity* (RP)! In encystment experiments with these doublets, both the global mirror-image pattern and the subregion anterior-posterior reversal emerged unchanged in excystment and were subsequently propagated (26).

Conjugations between double monsters and singlets that result in equivalency of the nuclear and cytoplasmic complements of the exconjugants have no effect on the doublet exconjugant. It is therefore very unlikely that cortical inheritance could be due to altered DNA sequences or DNA modification. Accordingly, cortical inheritance must be classed as extranucleic (Figure 1). However, more is involved than heritably stabilized activation/repression of morphogenetic genes or their products (as in the systems listed in lines 1, 2, and 3 of Table 1): A plausible explanation is that messages emanating from the grafted-on cell fragments modify an existing feedback system between the cortex and genes that control morphogenetic patterns (17, 22) to produce completely new structures, such as those demonstrating anterior-posterior polarity reversal and mirror-image morphology (see also Footnote b, Table

1). Further, the encystment-excystment experiments show that morphologically distinct preformed structures do not play a direct role in this presumed feedback loop.

Systems Based on Inherited Modification of DNA

SPECIFIC BASE-ANALOG-INDUCED DEMETHYLATIONS TRIGGER HERITABLE COMMITMENT TO DIFFERENTIATION IN A MOUSE EMBRYONIC CELL LINE Methylation of the cytosine of CpG dinucleotides in DNA seems to play an important role in the regulation of transcription in many eukaryotic systems (14, 29, 35). Methylation leads to changes in local chromatin configuration that, in turn, alter the accessibility of genes to regulatory proteins and their susceptibility to DNase I digestion (14, 38). Most commonly, methylation inhibits gene expression whereas demethylation leads to gene activation. Tissue-specific genes are nearly fully methylated in the germline; the demethylation of these genes usually takes place only in the specific tissue of expression (14, 82). Genes introduced into the zygote by the father's sperm exhibit a male-specific methylation pattern distinct from the pattern of their alleles contributed by the mother's ovum (59). Apart from specific remethylation of the chromosomes in the germline and the demethylation of particular genes in differentiated tissues, methylation patterns are fairly stably inherited. The agency responsible for this heritability is thought to be a "maintenance methylase" that, during DNA replication, promptly adds methyl groups to the nascent daughter strand at sites corresponding to methylated sites in the template strand (29). In analogy to inheritance of base sequences, the inheritance of DNA methylation may thus be guided by a kind of enzyme-mediated complementarity. Inheritance mediated by substituents of DNA or by nucleic acid configurations other than polynucleotide sequence has been called "epinucleic" inheritance (46; Figure 1).

In the cytidine analog 5-azacytidine, a nitrogen atom is substituted for a carbon atom at the site of methylation of cytosine. The analog can replace cytosine in DNA synthesis and is a powerful inhibitor of DNA transmethylase (36). Cells treated with 5-azacytidine have a lower level of 5 methyl cytosine (5-mc) in their DNA than untreated cells. This loss of 5-mc is accompanied by activation of specific genes (29; See Table 1, footnote c).

Konieczny & Emerson (39) studied an example of heritably stable gene activation following transient treatment of cells with 5-azacydine, using the mouse embryonic cell line C3H 10T1/2, clone 8. This cell line has a fibroblastic morphology and is sensitive to density inhibition of growth in culture (i.e. it is permanent but not neoplastic).

An illustrative experimental protocol follows: C3H 10T1/2 cells were inoculated to a density of 50 cells per plate and treated with $3\mu\text{M}$ 5-

azacytidine for 20–24 hrs. The agent was then washed off and clonal growth continued for 2–4 weeks. Cells in 1500 colonies were then examined for altered morphology. In 7% of the colonies a mixture of adipocytes (fat storage cells) and morphologically unaltered cells was found; 25% of the colonies contained typical myocytes (muscle cells), and 1% contained chondrocytes (matrix-producing cells)—the remaining 67% of the colonies did not contain differentiated cells. Control clonal and mass cultures of IOT1/2 cells not treated with 5-azacytidine did not contain any cells of the 3 above-mentioned mesodermal phenotypes (estimated frequency $< 10^{-8}$).

Since none of the 5-azacytidine-treated clones consisted entirely of differentiated cells, it was inferred that the key demethylation events had produced stem cell lineages that were developmentally determined but morphologically undifferentiated. Depending on the particular demethylated control site, the stem cell lines could later give rise to differentiated adipocytes, myocytes, or chondrocytes.

To examine this inference, 5-azacytidine-treated cells were grown into clones and surveyed daily for the appearance of differentiated morphotypes. After myofibers, for example, first appeared in a colony, undifferentiated cells of that colony were isolated, cloned, and cloned again. By this procedure, eleven myogenic, two adipogenic, and two chondrogenic clonal lines were isolated. After three subclonings, each produced clonal lines that differentiated only into a particular cell type (i.e. a particular demethylation produced a particular differentiation potential). The heritable potential to give rise to myocytes, for example, was followed for 150 cell doublings in several clones and was fairly constant in successive generations of these clones, but different in different clones. For example, one clone might give rise to about 50% myogenic colonies in each generation, another to about 97% myogenic colonies (39).

A SINGLE GROWTH CYCLE IN AN ALTERNATE BACTERIAL HOST RESULTS IN HERITABLE CHANGES IN CHARACTERISTICS OF BACTERIOPHAGE T2
The discovery by Luria & Human, in 1952 (51, 54), of host-induced modification of bacteriophage T2 inaugurated research in the field of DNA restriction-modification. Subsequently, Arber's clarification of the underlying phenomena led to the discovery of inherited site-specific DNA modification and of restriction endonucleases (2). (In modifying the home-grown DNA with specific glucosylation or methylation markings, bacteria can distinguish foreign DNA from their own and eliminate it through restriction (52). The rejection of foreign DNA is an isolation mechanism that can serve as a spur to speciation.)

Restriction-modification (rm) systems occasionally entail population-wide acquisition of new traits that are then inherited. One such example is the

loss of glucosylation of the DNA of phage T2. (See Table 1, line 6 and footnote d).

The observations are as follows: When bacteriophage T2 infect a culture of strain B from *E. coli* (an r^+m^+ strain possessing both restriction and modification systems), each bacterium produces a burst of 100–200 phages. All progeny phage, in turn, can initiate a new cycle of infection in strain B. By contrast, if phage from the same suspension infect a culture of the (r^-m^-) double mutant, *E. coli* B rgl/4₀, the emerging phage cannot form plaques on strain B. However, they can plate normally on strain B rgl/4₀, the strain on which they were grown, and also on *Shigella dysenteriae* (r^-m^+), a related bacterial species. A single cycle of replication in B rgl/4₀ host cells has evidently wiped out the ability of the phage to grow on strain B!

Why are phage grown on B rgl/4₀ unable to grow on strain B? In normal phage T2 DNA, all cytosine residues are replaced by hydroxymethylcytosine (HMC). Subsequently, nascent HMC DNA is modified by the addition of glucose to about 70% of the HMC residues. Much like methyl groups, the glucose molecules protect T2 DNA from degradation (restriction) by specific nucleases of strain B of *E. coli*. The DNA of phage grown in strain B rgl/4₀ is not glucosylated (due to the absence of uridine diphosphoglucose pyrophosphorylase in this strain) and is therefore degraded in strain B (21). Further, strain B rgl/4₀ is also deficient in its restriction mechanism, therefore the unglucosylated DNA remains intact and a crop of unglucosylated phage is produced (52, 68). The alternate host, *S. dysenteriae*, like B rgl/4₀, lacks restriction nuclease activity but does contain the glucosylation mechanism. Accordingly, a replication cycle of B rgl/4₀-grown T2 phage in *Shigella* restores glucosylation and therefore the ability to grow on the B strain.

Systems Based on Induced Loss of Nonessential Nucleic Acid (NA) Sequences

A considerable number and variety of systems have been studied in which loss of a stably inherited nucleic acid-containing element is induced by an environmental agent or treatment (see Table 1, lines 7, 8, and footnote e). Since they involve change in the nucleotide complement, such systems have been called nucleic systems in contradistinction to extranucleic systems (Table 1, lines 1–4) and epinucleic systems (Table 1, lines 5, 6) that entail no alterations in nucleotide sequence (46).

BLEACHING OF CHLOROPLASTS OF *EUGLENA GRACILIS* BY STREPTOMYCIN A suspension of *Euglena gracilis* was test-plated on solid media and gave rise to 100% green photosynthesizing colonies. Streptomycin at a concentration of 160 $\mu\text{g/ml}$ was then added and the suspension further incubated for 6 days. A fresh test-plating following this incubation now gave rise to

100% white (colorless) colonies. The cells constituting the white colonies lacked chloroplasts, whereas the parental green cells each contained about 10 chloroplasts. Colorless cells produced by streptomycin treatment were serially transferred for at least 10 generations in the presence of light in streptomycin-free media containing butyrate as a carbon source. No green cells appeared among the progeny (see Table 1, line 7). The rate of destruction of chloroplasts depends principally on the length of time of exposure to streptomycin and its concentration. Destruction is already discernible at $1\mu\text{g/ml}$. The concentration of streptomycin used for bleaching— $160\mu\text{g/ml}$ —does not affect the viability of *Euglena*. Bleaching takes place in both the presence and absence of light and is more efficient in growing cells (66, 67). *Euglena* can also be bleached by incubation at $34\text{--}35^{\circ}\text{C}$. Bleached *Euglena* are indistinguishable from flagellates of the genus *Astasia* (66).

HEAT-INDUCED ELIMINATION OF HERITABLY TRANSMITTED INFECTION BY VIRUS SIGMA IN *DROSOPHILA* Following a routine procedure for anesthetizing *Drosophila* through anoxia, L'Heritier & Teissier discovered a CO_2 -sensitive strain (48, 49). Flies of the sensitive strain failed to recover from a 30 second contact with CO_2 —they woke up from narcosis completely paralyzed and soon died. By contrast, normal resistant flies survive several hours of exposure to pure CO_2 . The trait of CO_2 sensitivity was due to infection by a rhabdovirus named sigma. (Single-stranded RNA-strand, approximately 4×10^6 dalton). About one third of the wild strains of *D. melanogaster* in France were CO_2 -sensitive. Sigma infection is not contagious but resistant strains can be rendered sensitive by injection of virus preparations into adult flies. So-called "stabilized" CO_2 -sensitive strains are produced by crosses between sensitive females and resistant males. Females of stabilized strains transmit sensitivity to virtually all their progeny. It is estimated that oögonia of stabilized females each contain 10–40 virus particles and that mature oocytes contain about 10^6 particles each. Sigma is therefore carried through the germline of *Drosophila* by abundant infection of the oocyte cytoplasm and *not* by association with the chromosomes. Males of stabilized strains transmit sensitivity only sporadically, presumably because sperm contain less virus than eggs. CO_2 sensitivity therefore shows a predominantly maternal-inheritance pattern.

Transmission of CO_2 sensitivity in stabilized strains can be cut off completely by keeping egg-laying females for about 6 days at 30°C or above. All the progeny of such females is CO_2 -resistant (see Table 1, line 8). Similarly, when spermatogenesis takes place in males held at about 30°C their spermatozoa no longer transmit CO_2 sensitivity (10, 65). (By contrast, if mature spermatozoa are kept at about 30°C , their ability to transmit sigma is not impaired.)

Systems Based on Acquisition of Foreign Nucleic Acid

In the preceding section we discussed heritable changes triggered by chemically or physically induced mass eliminations of elements containing nucleic acid (NA). Obviously, all the heritably transmitted NA elements susceptible to such selective curing had been acquired earlier by their host organisms. The elements include double-stranded DNA and RNA and single-stranded RNA and range in size from bacteria to small viruses (Table 1, lines 7, 8, and footnote e; Figure 1); some are lodged in the nucleus, others in the cytoplasm.

Several cases are known where the process obverse to curing—the acquisition of particular NA-borne gene banks—occurs with near 100% efficiency. Examples are: the high frequency transduction into *E. coli* of gal genes by the phage vector λ dg (61); conjugal transfer of the plasmid F (47) and of F' plasmids (sexduction) (1); ordinary lysogenizations (55) and phage conversions with toxigenic phage (4). These cases of *mass acquisition of particular NA elements clearly conform to our operational definition of IAC*. Once such specific cases of acquisition (“pick-up”) of foreign DNA are recognized as instances of IAC, one is led to ask whether the *operational definition of IAC* should not be expanded to include all cases of acquisition of foreign NA, regardless of whether the acquisition is a frequent experimentally controlled event or a rare occurrence inferred from molecular studies, e.g. acquisition of the mitochondrial precursor prokaryote by a host cell perhaps one billion years ago (13).

This more inclusive perspective of IAC is a logical outgrowth of the

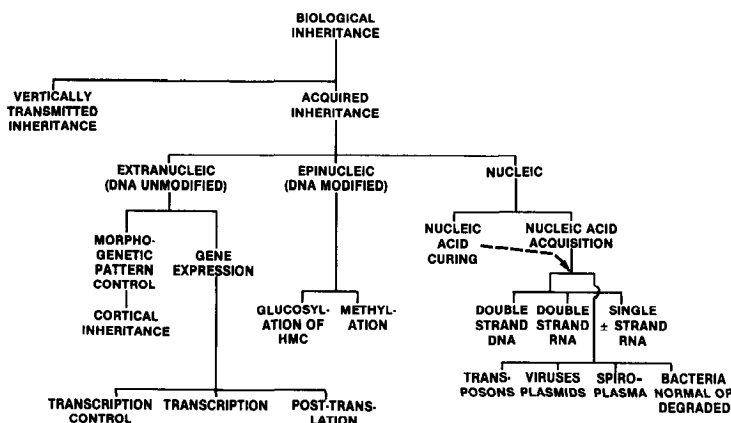


Figure 1 Characterization of IAC systems, their relationships to Mendelian inheritance (34) and to each other (46; J. Lederberg, personal communication). All DNA or RNA elements subject to curing were acquired earlier by the host (arrow). The inclusive category “biological inheritance” implies exclusion of cultural inheritance.

revolutionary discoveries, since 1950, concerning the existence, origin, and biological role of such nonchromosomal gene-banks as plasmids, viruses, and degraded or intact bacteria (and transposon- or virus-derived chromosomal genes).

Inheritance of acquired NA elements is a well-established phenomenon and discussion of the prototype systems shown in lines 9 and 10 of Table 1 is therefore omitted. The rationale for selecting the particular systems of lines 9 and 10 is as follows: The R plasmid system (line 9) exemplifies (horizontal) transmission of an ubiquitous, large DNA gene bank to extremely diverse recipients in many families of gram⁻ and gram⁺ bacteria (27, 72, 77). The retrovirus system sketched in line 10 illustrates passage of an acquired NA element through the germ lines of succeeding generations of mice (32). (It has been estimated that 0.5% of DNA in the mouse genome is of retroviral origin (80)—enough DNA to code the information of about 25,000 genes.) Other examples of acquired NA elements carried through the germline are: The mitochondrial defect “poky” of *Neurospora* (58); the male-killing spiroplasma “sex ratio” of *Drosophila* (81); the bristle abnormality, “S”, of *Drosophila* due to a reovirus (segmented DS RNA) (50); “P/M hybrid dysgenesis” in *Drosophila*, carried by mutagenic transposons called P elements (70).

IAC IN EVOLUTION AND EVOLUTIONARY SALTATION

Before Darwin, evolutionary thinking was dominated by Lamarck’s idea that inherited characteristics changed adaptively under the influence of changing environments. In the present paper it has been shown that some characters are indeed changed heritably in response to environmental influences but only some of the acquired traits are adaptive (For example: Penicillin-induced L forms or protoplasts are more penicillin-resistant than bacilli (43); yeast cured of their mitochondria by azide are no longer sensitive to this agent (62); lysogenic or plasmid-carrying bacteria are immune to superinfection by the homologous agents). With respect to adaptiveness, the acquired characters thus resemble random mutations and, like mutations, are subject to natural selection. However, there is little doubt that, as a class, the acquired changes differ from mutations: As organisms acquire entire integrated gene banks, a notable proportion of them achieve major evolutionary advances “saltations” (24)—or evolution by sudden spurts (18). Ancient examples are the acquisition of the precursors of mitochondria and chloroplasts (13); a contemporaneous example is the acquisition of the R26 plasmid that conveys multiple antibiotic resistances and versatile conjugational capabilities (72).

These examples show that a clearcut line can be drawn between NA elements “horizontally” acquired from the environment or from *unrelated*

species and “vertical” heredity, *The presence of identical genes in ancestors and descendants*, according to Johanssen’s classical definition (34).

IAC systems governed by extranucleic mechanisms also suggest possible examples of saltatory evolutionary events. One such example is the previously described appearance of bilateral symmetry in doublets of the ciliate *Pleurotricha lanceolata* (26). Another extranucleic IAC system that might have served as a point of departure for evolutionary saltation is the protoplast L-form system of bacteria. Protoplasts and L forms are unable to divide in liquid media; they are osmotically fragile (40, 43) and their ability to interact with phage and naked DNA is entirely changed (31, 57). Mutants are rapidly selected to compensate for these defects—including the block in cell division (41), membrane fragility, and other membrane properties. Further, the naked state greatly increases the probability of cell fusion and consequent major alterations in gene function (28, 44). Thus, one may speculate that an L-form-like state may have supervened as a transitional stage between prokaryotes and naked incipient eukaryotes.

RETROSPECT AND PROSPECT; SUMMARY

Space limitations oblige us to omit a review of the stormy historical debates concerning IAC and also of the relationship between IAC and differentiation. Further, we omit speculation on the directions that the study of IAC systems may take in the future.

Transitory treatment by physical, chemical, or biological agents of certain single-cell systems or multicellular organisms may induce in them—en masse—changes in particular characteristics that are then heritably transmitted. The molecular basis of this altered inheritance is quite different in the different systems under discussion. In some, gene expression was stabilized at a new equilibrium or in a new morphogenetic pattern (extranucleic). In others, DNA modifications have taken place (epinucleic). In a third set of systems, loss of a nonessential DNA or RNA element may have been induced or acquisition of a new NA element may have occurred (nucleic).

Acquired characteristics of the extranucleic, epinucleic, or nucleic types may become heritable in single-cell systems and some of these heritable changes may be adaptive. Apparently, however, only characters of the nucleic type can pass the germline of multicellular organisms.

Major evolutionary saltations resulted from the (horizontal) acquisition of gene banks; other saltations may have originated in such extranucleic heritable states as bacterial L forms or double monsters of protozoans.

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