the basis of this precedent, the F and R groups of equine herpesvirus should be distinguished taxonomically. We propose that DNA restriction endonuclease patterns of the F viruses be designated as prototypic of equine herpesvirus 1, whereas those of the R viruses be designated as prototypic of equine herpesvirus 4, in accordance with the recommendations for naming new herpesviruses by the Herpesvirus Study Group of the International Committee for Taxonomy of Viruses (*13*).

Our studies demonstrate the segregation of related viruses of the same host into distinct species on the basis of restriction endonuclease analyses of their DNA's. The emphasis on the DNA fragment patterns exemplified by the viruses analyzed in this study as the basis for classification reflects our prediction that F viruses may occasionally cause respiratory illnesses and that, conversely, R viruses may occasionally be responsible for sporadic abortions, in a manner analogous to the overlap in the human disease patterns and sites of isolation of herpes simplex viruses 1 and 2. The segregation of these viruses into distinct groups strengthens the previous reports suggesting biologic and possibly immunologic differences between R and F viruses and indicates that they should be treated as epidemiologically and taxonomically distinct infectious agents.

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Induction of Invagination in Insect Epithelium: Paradigm for Embryonic Invagination

Abstract. *The proposal that adhesive disparities between inpocketing populations of cells and surrounding epithelia drive epithelial invagination was tested in grafting experiments with moth pupal wing epithelium. Evidence exists that a cellular adhesiveness gradient spans the proximodistal axis of the wing. Although pupal wing cells normally do not invaginate or evaginate, epithelial folding can be induced after exchange of grafts from opposite ends of the proximodistal axis. The hypothesis that cytoskeletal elements are the primary agents in epithelial invagination should be reevaluated.*

Embryonic form is molded as epithelial cell sheets repeatedly fold inward and new cell arrangements arise. Initial inpocketing of epithelia is characterized by a localized thickening of the cell sheet (placode formation) that entails epithelial cell elongation and an increase in lateral contacts of the cells. Inpocketing of this thickened placode proceeds as epithelial cells broaden at their basal poles and assume wedge-shaped forms (*1*). Although the prevailing view attributes changes in cell shape to microtubule elongation and contraction of apical microfilaments, the increase in intercellular contact area that usually accompanies epithelial inpocketing led to the idea that localized changes in cellular adhesiveness might govern inpocketing of cell populations during embryonic development (*2*). Ideally, the soundness of the latter idea would be tested by exchanging small populations of cells in an undifferentiated tissue whose various regions differ quantitatively in adhesive properties. In insect epithelia, gradients of cellular adhesiveness appear to exist in single tissues (*3*, *4*). Numerous grafting experiments support the idea that a proximodistal gradient of cell adhesiveness spans the pupal wing epithelium of *Manduca*, the proximal cells of the wing being more adhesive than the distal cells (*4*). Since either invagination or evagination can result after exchange of cell populations between distal and proximal extremes of the pupal wing, support is gained for the proposal that adhesive properties of cells participate in epithelial folding.

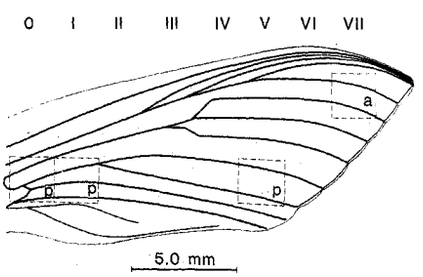

Fig. 1. Pupal wing of *Manduca*. Each region of the wing is assigned a position along a proximodistal axis (numerals *0* to *VII* at top of figure) as well as a position along the anteroposterior axis (*a* or *p*). Proximal is at left; posterior is at bottom. Regions bounded by dashed lines (approximately 2 by 2 mm areas) were chosen for grafting. Heavy lines represent tracheae. They are predominantly aligned along the wing's proximodistal axis and were used as landmarks during grafting.

The moth pupal wing consists of an upper and lower epithelial layer separated by a matrix of proteoglycans and collagen (*5*, *6*). Nuclei and intercellular junctions are located primarily at the apical poles of cells, and the cells taper toward their basal poles. Epithelial cells form cuticle on their apical surfaces and a basement membrane on their basal surfaces. Grafts were exchanged on the upper layer only (Fig. 1). Adult development begins about 2 days after pupation when epithelial cells retract from the overlying pupal cuticle. Results were scored when adult moths emerged 3 weeks after pupation. Details of surgical manipulations and animal culturing are described in (*4*).

Normally, the exchange of epidermal grafts in the wing does not alter the planar arrangement of epithelial cells (*4*). However, grafts (2 by 2 mm) exchanged between proximal (supposedly more ad-

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hesive 0p and Ip) and distal (supposedly less adhesive Vp and VIIa) extremes of the wing did circularize and fold in a small percentage of cases (Table 1). Only grafts that had been transposed to more distal positions exhibited invagination. Grafts that were transposed proximally showed a high frequency of evagination, but never showed invagination. These observations suggested that invagination of wing epithelium occurs in those cells whose adhesiveness exceeds that of surrounding epithelial cells. Other possible explanations for folding movements have been considered (*7*).

Quantitative differences in adhesiveness can account for the circularization of grafts whose initial outlines are square; these differences, if sufficiently great, can result in further reduction of the graft-host interface. Once the limits of compressibility within the plane of the epithelium have been reached, the graft must fold under the pressure of lateral forces. Despite the tendency of less adhesive cells to maximize their contacts with more adhesive cells, the latter cells should increase their homotypic contacts at the expense of contacts with adjacent cells that are less adhesive. Hence, the equilibrium conformation adopted by the two interacting populations should be determined by rearrangement of cells (*8*).

Forms assumed by graft cells support the idea that quantitative differences in adhesiveness are involved in the folding of insect epithelia. Sections of Ip grafts that had been placed at the distal extreme of the wing revealed that, relative to untransposed epithelia, proximal cells underwent marked changes in form. Elongation of cells within the plane parallel to the surface is localized at the periphery of the graft (Fig. 2C). Here only tenuous heterotypic contact is established with host cells. In the interior of the graft, cells have stretched along their apicobasal axes and increased their homotypic lateral contacts (Fig. 2, A and B). With nuclei of graft cells located primarily in the basal half of the epithelium, cells of distally transposed grafts assume wedge-shaped forms, predisposing them to buckle inward as a coherent sheet under lateral tension arising from cellular rearrangements.

Proximally transposed grafts (VIIa and Vp → 0p and Ip) buckle outward with a high frequency; in a few cases, the grafts form isolated vesicles on the apical surface of the host epithelium. Contact of cells along their apicobasal axes is augmented, but only after the sheet of cells has folded out. Folding is initiated while nuclei still occupy apical positions, and cells are broadest at the apical pole. Presumably these morphogenetic changes occur in response to tangential adhesive forces acting at the graft-host interface.

If tangential forces at the graft-host interface drive the morphogenetic changes in pieces of displaced epithelium, the total force exerted per unit of apical surface area around the periphery of the graft should increase as the apical surface area of the graft diminishes. As apical surface area decreases geometrically, lateral surface area decreases arithmetically. The low frequency of invagination associated with 2 by 2 mm

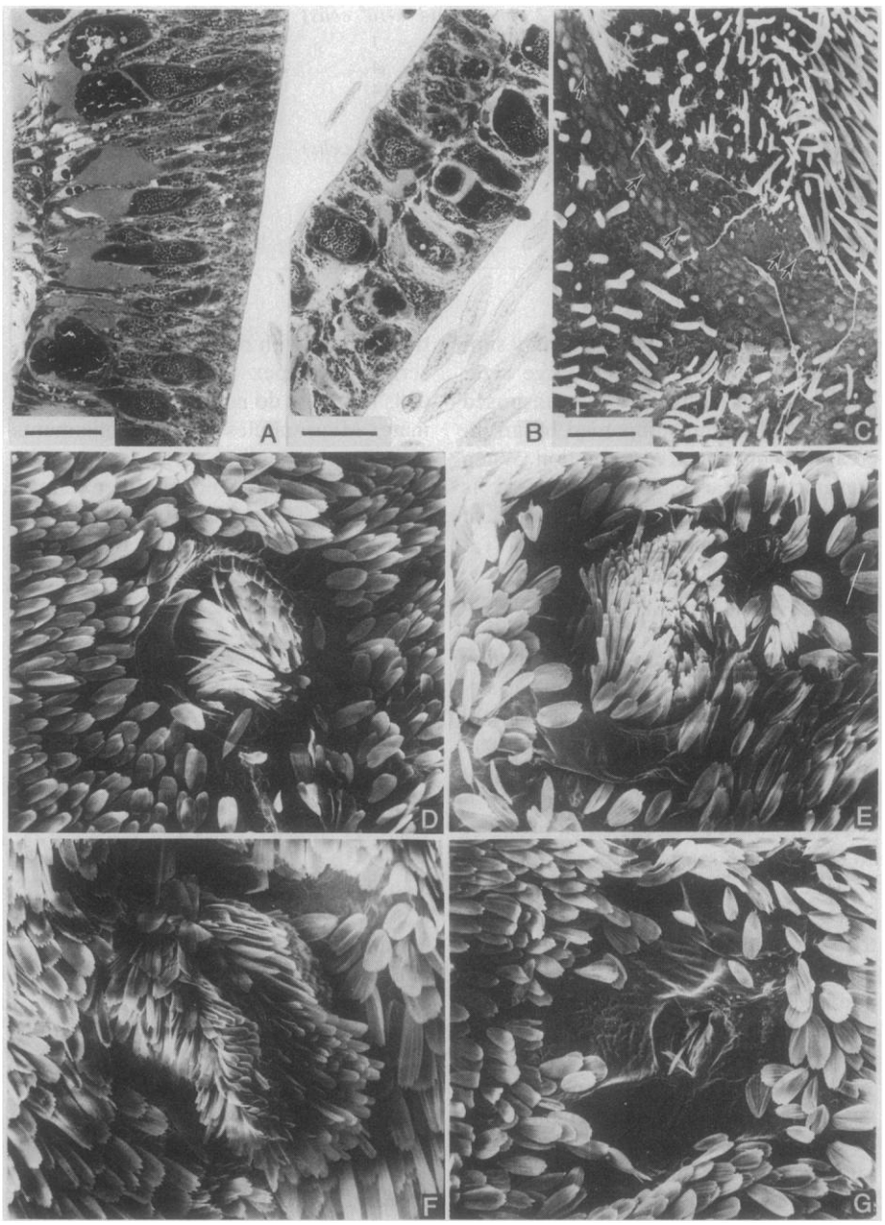

Fig. 2. (A to C) Forms assumed by wing epithelial cells. All tissues were fixed 7 days after pupation in cacodylate-buffered glutaraldehyde (3 percent). Specimens for light microscopy (A and B) were sectioned at 1 μm. Tissue in (C) was critical-point-dried and coated with gold-palladium for scanning electron microscopy. (A) Section of a Ip graft transposed to region VIIa. Note the marked elongation of cells. The basement membrane between upper and lower epithelial layers is marked with arrows. (B) Normal arrangement of cells in the two epithelial layers of the wing. (C) Surface view of interface between a Ip graft (upper right) and VIIa host (lower left). Note elongated cells (single arrows) around graft perimeter and the relatively small area of exposed surface for cells in the interior of the graft (double arrows). Bars in (A) and (B) represent 25 μm; bar in (C) represents 100 μm. (D to G) Representative scanning electron micrographs of invaginated and evaginated adult wing epithelium. All grafts were 1 mm^2. Proximal is to the left; anterior is at the top (×60). Graft scales are longer and narrower than host scales in (D), (E), and (G). Size and form differences in host and graft scales are not as obvious in (F). (D) Inpocketing of Ip at Vp. (E) Invagination of Ip at VIIa. (F) Evagination of VIIa at Ip. (G) Invagination of 0p at VIIa. The graft has almost completely segregated from host cells and only a few scales protrude from the inpocketing.

Table 1. Invagination and evagination of transposed wing grafts. In scoring configurational changes of grafts, all transposed epithelia that occupied a position below the plane of the surrounding host epithelia were considered to be invaginated and those above to be evaginated. Variations occurred, from slight graft inpocketings and outpocketings (Fig. 2, D to G) to complete segregation of graft from host tissue. In the latter, a vesicle either formed between upper and lower epithelial layers of the wing (invagination) or was found lying free on the apical surface of the upper epithelial layer of the host (evagination).

Graft-host combination	Number of exchanges	Percentage of grafts (0p and Ip) that invaginated	Percentage of grafts (VIIa and Vp) that evaginated
	2 by 2 mm graft (4×10^4 cells)		
Ip ↔ VIIa	28	7	43
Ip ↔ Vp	26	4	38
0p ↔ VIIa	14	14	43
0p ↔ Vp	15	20	87
	1 by 1 mm graft (1×10^4 cells)		
Ip ↔ VIIa	16	56	81
Ip ↔ Vp	18	58	83
0p ↔ VIIa	12	100	100
0p ↔Vp	11	100	100

grafts moved to distal positions may simply be attributable to their large size; further decreases in size of transposed grafts should be accompanied by an increased frequency of invagination. When the apical surface area of the graft is reduced by one-quarter, the frequency of invagination is markedly increased (Table 1 and Fig. 2, D and E).

The frequency of invagination should be further accentuated by transposition of tissue from the more proximal region 0p to VIIa and Vp, since adhesive disparity between cell populations is intensified as the distance separating them along a proximodistal adhesiveness gradient increases (Table 1 and Fig. 2G).

The otic placode of the chick occupies an area of 60,000 μm^2; other embryonic placodes are comparable in size (*9*). Since cellular dimensions for wing epithelial cells match those for cells of vertebrate epithelia, we can estimate that hundreds, rather than thousands, of cells are usually involved in invagination. Below a certain size, all distally transposed wing grafts from region Ip may invaginate (Table 1). A corollary claim would be that adhesive disparity between placode and surrounding epithelium should increase as dimensions of an invaginating region increase.

Microtubules and microfilaments are generally accepted as the agents responsible for changes in cellular form during invagination (*1, 10*). The contention that cytoskeletal elements are the primary forces behind folding movements has been based mainly on the use of drugs that, although they disrupt microtubules and microfilaments, also affect the cell surface (*7*). Even though all cells in wing epithelia of pupal and developing adult moths are richly endowed with microtubules oriented along the apicobasal axis of the cell and with microfilaments circumscribing the apex of the cell (*11*), these cells normally do not invaginate or evaginate. Microtubules and microfilaments may simply stabilize forms adopted by cells under the influence of other cellular forces (*12*). These forces could be generated by adhesive properties of cell surfaces as cells adopt configurations of minimum free energy (*8*).

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The Complement System of the Nurse Shark: Hemolytic and Comparative Characteristics

Abstract. *The complement system of the nurse shark was investigated. Six functionally pure components were isolated from a single serum sample. Sequential reactions of the components with sensitized sheep erythrocytes resulted in membrane lesions indistinguishable from the "holes" caused by guinea pig complement.*

The nurse shark, *Ginglymostoma cirratum*, has survived 350 million years of evolution. We have separated from the serum of this ancient species six functionally pure complement components (*1*); their sequential interactions result in the lysis of sensitized target cells. We present evidence for the existence of this six-component system and for the classical pathway (*2*) of its activation, which results in membrane lesions that are indistinguishable from the "holes" produced by mammalian complement.

Naming components of a complement (C) system, shorter than the nine-component mammalian one, was problematic because numbers or letters imply analogies that may not be appropriate. In the past, we used "n" to indicate nurse shark origin; thus, nurse shark antibody was designated An and sheep erythrocytes sensitized with that antibody were designated EAn. In 1973 we isolated, purified, and characterized the first component of the system and called it C1n (*3*).

Subsequently, we found that the mammalian intermediate complex, EAC1–7, could be lysed by two individually inactive serum fractions which we assumed to be the preterminal and terminal components of the shark system. After their isolation and purification we called them accordingly t1 and t2 (*4*). Three additional components, X, Y, and Z, discovered more recently, reacted after C1n and before t1. The reaction sequence of the six components was C1n-

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