anism that shapes oral protrusions (jaws and lips) preceded evolution of jaws (Fig. 4). In some of the Paleozoic agnathans, movable oral plates and lips are thought to be very similar to jaws at the phenotypic level (*20*). However, there are no morphologic homologies (*1*) between jaws and lips that can satisfy both the gene expression patterns and the topographic relationships of the structures simultaneously. Rather, the shared molecular mechanisms are regarded as exaptations (*21*) for jaw evolution and not as a guide for homology. We conclude that a topographical shift of epithelial-mesenchymal interaction lies behind this difference.

As inferred by the present study, the difference in developmental patterning may partly be due to the caudal restriction of growth factors in the epidermis (Fig. 4, top). Haeckel (*22*) has defined such a change in the place of development as "heterotopy." In organisms such as vertebrates, in which epigenetic tissue interaction plays an essential role in morphogenetic patterning, the shift of epigenetic interactions alters the regulatory gene expression patterns in the mesenchyme (*9*), which leads to reorganization of tissue morphological identities. Thus, the gene cascades and morphological homology are uncoupled through the process of heterotopy. In this sense, the gnathostome jaw is truly an evolutionary innovation, which appears to have been obtained by overcoming ancestral developmental constraints (*23*). What brought about this shift in the regulation of the growth factor–encoding genes requires future study.

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13. Heparin beads (Sigma) soaked with mouse FGF8b (500 μg/ml; R&D Systems, Minneapolis, MN) and human BMP4 (500 μg/ml; R&D Systems) were implanted in the embryonic lamprey head. After 2 days, the embryos were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). For the chick embryo, the same recombinant beads were implanted in both sides of the postoptic region of seven-somite embryos.
14. Embryos of *L. japonica* were injected with a solution of DiI (Molecular Probes, Eugene, OR) as described in (*3*). Embryos were then incubated 24 to 48 hours and fixed with 2% PFA containing 1.25% glutaraldehyde in PBS and then cleared with 80% glycerol for observation by fluorescence microscopy.
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fig. S1

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Induction and Suppression of RNA Silencing by an Animal Virus

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RNA silencing is a sequence-specific RNA degradation mechanism that is operational in plants and animals. Here, we show that flock house virus (FHV) is both an initiator and a target of RNA silencing in *Drosophila* host cells and that FHV infection requires suppression of RNA silencing by an FHV-encoded protein, B2. These findings establish RNA silencing as an adaptive antiviral defense in animal cells. B2 also inhibits RNA silencing in transgenic plants, providing evidence for a conserved RNA silencing pathway in the plant and animal kingdoms.

Posttranscriptional gene silencing, quelling, and RNA interference (RNAi) are mechanistically related RNA silencing processes that destroy RNA in a sequence-specific manner (*1*, *2*). Available data show that double-stranded RNA (dsRNA) serves as the initial trigger of RNA silencing and, after recognition, is processed by the Dicer RNase into short fragments of 21 nucleotides (nt) in length. These short interfering RNAs (siRNAs) are then incorporated into a dsRNA-induced silencing complex (RISC) to guide cycles of specific RNA degradation (*1*, *2*). Here, we report that RNA silencing plays a natural antiviral role in animal cells, as has been established in plants (*3*, *4*).

We focused on the flock house virus (FHV) because its *B2* gene (see fig. S1) shares key features, but not sequence similarity, with the plant cucumoviral *2b* gene (*5*), which encodes a known group of silencing suppressors (*6*, *7*). Both open reading frame (ORF) 2b and B2 overlap the carboxyl terminal region and occupy the +1 reading frame of the ORF encoding the viral RNA-dependent RNA polymerase and are translated in vivo by a subgenomic mRNA (*5*).

The FHV B2 protein indeed exhibited a potent silencing-suppression activity (Fig. 1) in the *Agrobacterium* co-infiltration assay (*8*), established in transgenic plants that express green fluorescent protein (GFP). Transient B2 expression prevented RNA silencing of the GFP transgene, leading to a strong and prolonged green fluorescence examined under ultraviolet (UV) illumination (Fig. 1, left), similar to suppression by the cucumoviral 2b proteins (*9*) (Fig. 1, right). In contrast, a broad red fluorescent zone surrounding the infiltrated patch (Fig. 1, middle) became clearly visible 6 days after infiltration, when the co-infiltrated transgene directed translation of neither 2b nor B2.

RNA blot hybridizations confirmed that expression of either protein was associated with high accumulation levels of the GFP mRNA (see fig. S2). In addition, the GFP-specific siRNAs, a hallmark of RNA silencing (*10*), remained at extremely low levels in the leaves where there was expression of either B2 or 2b (see fig. S2). We further demonstrated that B2 was able to functionally substitute for 2b of cucumber mosaic virus (CMV) in whole plant infections (see methods sections of online ma-

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terial), as found previously for a CMV 2b homolog (*11*). B2 suppression of RNA silencing in plants explains why FHV is able to overcome the RNA silencing defense and establish systemic infections in transgenic plants that express a plant viral protein that facilitates virus cell-to-cell movement (*12*).

Our finding that an FHV-encoded protein suppresses RNA silencing in plants suggests a role for RNA silencing in FHV infections of animal hosts. FHV belongs to the *Nodaviridae* family, members of which naturally infect vertebrate and invertebrate hosts, and *Drosophila* cells support complete infection cycles of FHV (*13*). We found that infection of *Drosophila* S2 cells with FHV virions resulted in a rapid appearance of the FHV-specific siRNAs of both positive (Fig. 2A) and negative polarities. Accumulation of the siRNAs trailed that of FHV genomic and subgenomic RNAs (Fig. 2C), which suggests that the decreased accumulation of FHV RNAs at later stages of FHV infection (*14*) (Fig. 2B) may be caused by an FHV-specific RNA silencing.

To investigate this possibility, we constructed a full-length FHV RNA1 cDNA clone (pRNA1) (see methods sections of online material), which, after transfection into S2 cells, directed RNA1 self-replication and transcription of RNA3 (*15*), the subgenomic mRNA for B2 (Fig. 2D, lane 2). We found that depleting the mRNA for Argonaute2 (AGO2) by RNAi, an essential component of the RISC complex (*16*), led to a pronounced increase (two- to threefold) in the accumulation of FHV RNAs 1 and 3 (Fig. 2D, lanes 6 to 8), whereas co-transfection of cyclin E or GFP dsRNAs with pRNA1 had minimal effect (Fig. 2D, lanes 4 and 5), indicating that a functional RNA-silencing pathway naturally restricted FHV accumulation in the host cells. Furthermore, co-transfection of pRNA1 with a dsRNA targeting the 3′-terminal 500 nucleotides of FHV RNA1 completely prevented the accumulation of intact FHV RNA1 in S2 cells (Fig. 2D, lane 3). These results collectively demonstrate that FHV is both an initiator and a target of RNA silencing in this animal host.

Further studies showed that B2 was essential for FHV accumulation in *Drosophila* cells, which is in contrast to a previous study carried out in nonhost mammalian cells (*15*). A B2-knockout mutant of FHV RNA1, referred to as RNA1-ΔB2 (see methods sections of online material), which contains the previously described point mutations (*15*) that converted the first and 58th codons of the B2 ORF into serine and stop codons, respectively, failed to accumulate to detectable levels after transfection into S2 cells (Fig. 2D, lanes 12 and 20). This defect was partially trans-complemented (up to 10% of the wild-type level) by co-transfection of a plasmid expressing either B2 (Fig. 2D, lanes 13 and 21) or a His-tagged B2 (Fig. 2D, lane 22). Expression of the His-tagged B2 from the co-transfected plasmid was detected in S2 cells by Western blot analysis using an antibody recognizing the His tag. Reverse transcription–polymerase chain reaction and sequencing revealed that the introduced mutations were stably maintained in the progeny FHV RNAs extracted from infected cells, indicating that B2 was indeed expressed from the co-transfected plasmid rather than from a revertant RNA1.

Accumulation of RNA1-ΔB2 in S2 cells was efficiently rescued, up to 50% of the wild-type level, by co-transfection with the AGO2 dsRNAs, either singly (Fig. 2D, lanes 14 and 15) or in combination (Fig. 2D, lane 16). However, co-transfection with dsRNAs targeting mRNAs of the two *Drosophila* Dicer genes (*17*) was not effective (Fig. 2D, lane 17) under the same conditions. This is possibly due to a more efficient mRNA depletion by RNAi for AGO2 (Fig. 2D, lanes 14 to 16) than for Dicer (*16*, *17*), which is required to process the input dsRNA. Notably, the level of complementation by RNAi of AGO2 (Fig. 2D, lanes 14 to 16) was higher than that achieved by the B2-expressing plasmid (Fig. 2D, lane 13), although was still achieved less efficiently than B2 expression from wild-type RNA1 (Fig. 2D, lane 10). Therefore, in the absence of B2 expression, FHV RNAs 1 and 3 accumulated to substantial levels when the RISC complex was disrupted by AGO2 depletion. These data confirmed the previous finding (*15*) that B2 is not required for RNA1 self-replication and indicate that the essential function of B2 for FHV infection of the S2 host cells observed in this study was to suppress RNA silencing that targeted FHV RNAs for degradation. Thus, the same protein blocks RNA silencing in both animals and plants, providing the first experimental evidence for a highly conserved RNA silencing pathway in different kingdoms.

It is known that RNA silencing operates in animals, including mammals (*1*, *2*, *18*). In this work, we demonstrate that infection of *Dro-*

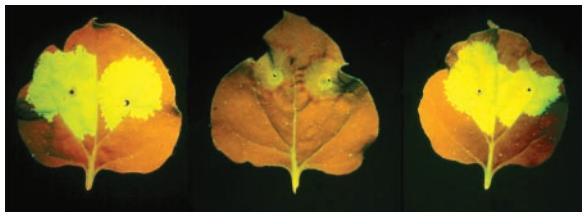

Fig. 1. Cross-kingdom suppression of RNA silencing in plants by an animal viral protein. The GFP-expressing *Nicotiana benthamiana* leaves were co-infiltrated with a mixture of two *Agrobacterium tumefaciens* strains, as described (*8*, *9*). One directs the expression of GFP and thereby induces GFP RNA silencing, and the other simultaneously expresses the FHV-encoded B2 (left leaf), B1 (middle leaf), or the plant cucumoviral 2b (right leaf). The leaves were detached and photographed under UV illumination 6 days after infiltration. GFP silencing is visualized in the middle leaf as a bright red color zone surrounding the infiltrated patch caused by chlorophyll fluorescence.

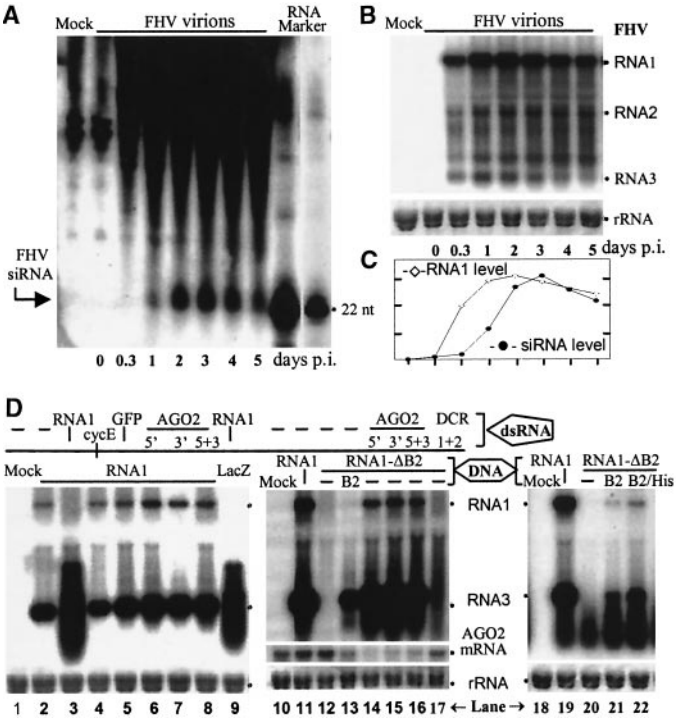

Fig. 2. Induction and suppression of RNA silencing in *Drosophila* by FHV. (**A** to **C**) A time course analysis is shown on the accumulation of FHV siRNAs (A) and RNAs 1 to 3 (B) in S2 cells infected with FHV virions, and densitometry measurements of the accumulation levels of FHV RNA1 and siRNA are shown in (C). An RNA marker of 22 nt in length transcribed in vitro was loaded in the right lanes (A). (**D**) Accumulation of FHV RNAs in S2 cells transfected with pRNA1 or pRNA1-ΔB2, with or without dsRNA. dsRNA corresponding to mRNA of cyclin E (cycE), GFP, and two fly Dicer (DCR) genes; to the 5′ and 3′-terminal 1000 nt of the AGO2 mRNA (*16*, *17*, *23*); or to the 3′-terminal 500 nt of FHV RNA1; and a B2-expressing plasmid that was co-transfected into S2 cells are indicated above each lane.

sophila cells with an RNA virus triggers strong virus RNA silencing and that the same virus is equipped with an effective silencing suppressor essential for infection. These data provide direct evidence that RNA silencing naturally acts as an adaptive antiviral defense in animal cells. The specificity mechanism of this adaptive defense is based on nucleic acid base pairing between siRNA and its target RNA (*1*, *2*) and thus is distinct from cellular and humoral adaptive immunity based on peptide recognition (*19*). A prediction from our work is that heterologous sequences inserted into a replicating virus genome will lead to the production of a population of siRNAs capable of silencing other viral and cellular RNAs in trans that are homologous to the insert. Indeed, recent studies showed that viral sequences inserted in alphavirus vectors give rise to virus resistance in mosquitoes, which is dependent on the inserted RNA sequence rather than on its protein product (*20*, *21*). It will be of interest to determine if RNA silencing also plays a role in observed protection against mammalian viruses, derived similarly from heterologous expression of RNA sequences from a replicating RNA virus vector (*22*).

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fig. S1

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Is Face Processing Species-Specific During the First Year of Life?

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Between 6 and 10 months of age, the infant's ability to discriminate among native speech sounds improves, whereas the same ability to discriminate among foreign speech sounds decreases. Our study aimed to determine whether this perceptual narrowing is unique to language or might also apply to face processing. We tested discrimination of human and monkey faces by 6-month-olds, 9-month-olds, and adults, using the visual paired-comparison procedure. Only the youngest group showed discrimination between individuals of both species; older infants and adults only showed evidence of discrimination of their own species. These results suggest that the "perceptual narrowing" phenomenon may represent a more general change in neural networks involved in early cognition.

At first glance the development of the ability to recognize faces appears to follow a typical trajectory: rapid change during infancy, followed by more gradual improvement into adolescence (*1*). This pattern contrasts with some aspects of language development. For example, speech perception is characterized by a loss of ability with age, such that 4- to 6-month-olds can discriminate phonetic differences that distinguish syllables in both their native and unfamiliar languages, whereas 10- to 12-month-olds can only discriminate the phonetic variations used in their native language (*2*, *3*). Here we describe a similar phenomenon for face recognition: Specifically, we demonstrate that 6-month-old infants are equally good at recognizing facial identity in both human and nonhuman primates, whereas 9-month-old infants and adults show a marked advantage for recognizing only human faces.

Nelson (*4*) has proposed that the ability to perceive faces narrows with development, due in large measure to the cortical specialization that occurs with experience viewing faces. In this view, the sensitivity of the face recognition system to differences in identity among the faces of one's own species will increase with age and with experience in processing those faces. By adulthood the extensive experience with human faces can be mentally represented as a prototype that is "tuned" to the face inputs most frequently observed (human faces), with individual faces encoded in terms of how they deviate from the prototype (*5*). Because infants begin to show evidence of forming face prototypes by 3 months of age (*6*), their face recognition should become more "human face specific" some time after this. This leads to the prediction that younger infants, who possess less experience with faces than older infants and adults, should be better than older infants or adults at discriminating between individual faces of other species.

This hypothesis is indirectly supported by several lines of research. For example, human adults are far more accurate in recognizing individual human than monkey faces; the opposite is true for monkeys (*7*). Such species-specificity may be due to the differential expertise in the two groups: monkeys are more familiar with monkey than human faces, whereas humans are more familiar with human than monkey faces. Human infants, of course, likely have no experience with monkey faces and relatively little experience with human faces. This may confer upon them a more broadly tuned face recognition system and, in turn, an advantage in recognizing facial identity in general (i.e., regardless of species). This prediction is supported by a preliminary study (*8*) in which it was demonstrated using event-related potentials (ERPs) that young infants, but not adults, could discriminate monkey face identity across changes in facial orientation. A second ERP study examined the influence of stimulus inversion, a manipulation that in behavioral studies impairs adults' recognition of identity of human faces

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