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22. The patterns of β-galactosidase expression were determined on whole flies and on frozen sections from 3- to 5-day-old male and female flies of *PGAL4 UAS-lacZ* strains, with at least 10 flies per genotype. Abdominal structures that show a reproducible *GAL4* expression in male adult flies are the midgut (all strains); the oenocytes (strains A through E); the testis, the anterior ejaculatory duct, or the male accessory glands (strains A, B, D, and F; E and G showed both); the crop (C, E, F, and G); the Malpighian tubules (A, E, and G); the fat body (A and G); and the nephrocytes (D). There is also some variable and nonoverlapping *GAL4* expression in thoracic muscles and in neurons in the thorax and the head. LacZ expression was also detected in the salivary glands of all strains, including very weak expression in the control *UAS-lacZ* strain.
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34. Flies from the three control strains [*55B-GAL4* as subject males; Canton-S males and shibire (shi) females as objects] were chosen for their clear behavioral phenotype (*8*). With the *PGAL4 UAS-tra* males (of strains A through E), more than 60% of the *55B-GAL4* males showed sustained wing vibration (40% with strain E), more than 50% showed licking (30% with strain E), and 20 to 50% attempted copulation (10% with strain D). With males of the four control strains, 4 to 12% of *55B-GAL4* males yielded wing vibration (20% with G-*tra*), less than 5% showed licking, and 0% attempted copulation. *55B-GAL4* males showed 95%, 80%, and 65% of these behaviors with target shi females.
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37. We thank A. Brand, N. Perrimon, R. F. Stocker, and G. Technau for the *PGAL4* strains (A = 29B, B = 1407, C = OK72, D = 323-CyO, E = OK376, F = 55B, and G = 24B); J. Connolly, J. Keane, K. Moffat, and S. Sweeney for generating strains; J. M. Belote for antibodies to *tra*; G. J. Blomquist, F. M. Butterworth, J. A. Coyne, and F. Romer for comments; and M. Cobb for help with the manuscript. Supported in part by the Human Frontiers Science Program grant RG 93/94 (J.-F.F., F.S., and R.J.G.), by a fellowship from the Ministry of Education and Research (G.S.), and by grants from the Wellcome Trust (034320/Z/91/Z) and the European Union (ERBSC1*CT920790) (C.J.O'K.).

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A Similarity Between Viral Defense and Gene Silencing in Plants

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Gene silencing in plants, in which an endogenous gene is suppressed by introduction of a related transgene, has been used for crop improvement. Observations that viruses are potentially both initiators and targets of gene silencing suggested that this phenomenon may be related to natural defense against viruses. Supporting this idea, it was found that nepovirus infection of nontransgenic plants induces a resistance mechanism that is similar to transgene-induced gene silencing.

It has been shown that gene silencing (*1*) and virus resistance are related phenomena in transgenic plants. Transgenes that are derived from viral cDNA and are able to induce gene silencing may also suppress the accumulation of viruses that are similar in nucleotide sequence (*2*). In addition, nonviral transgenes are able to suppress virus infection if the virus is modified by insertion of the transgene sequence into the viral genome (*3*).

Viruses are also able to silence host genes. For example, in *Nicotiana benthamiana* inoculated with modified tobacco mosaic tobamovirus (TMV) (*4*) or potato X potexvirus (PVX) (*5*) that carried host-related inserts, there was suppression of genes homologous to the inserts. Viruses can also induce silencing of transgenes that are similar in sequence to the inoculated virus (*6*). Early in the course of infection, expression of the transgene was unaffected by the virus, and the normal viral symptoms were produced. However, later on, in the upper leaves that developed after the virus had spread systemically, gene silencing affected both the transgene and the homologous virus. Thus, leaves that developed later contained lower concentrations of the transgene RNA, were free of the virus, and were resistant to secondary infection by the virus. The plants exhibiting this response were said to have "recovered" (*6*).

This type of recovery from virus disease is not confined to transgenic plants. In nepovirus-infected *Nicotiana* sp., there are severe viral symptoms on the inoculated and first systemic leaves. However, the upper leaves that develop after systemic infection are symptom-free and contain a lower concentration of virus than do the symptomatic leaves (*7*). For example, *N. clevelandii* inoculated with tomato black ring nepovirus (strain W22) initially shows symptoms and later recovers (Fig. 1). After secondary reinoculation of W22 to the recovered leaves, there was no additional accumulation of W22 RNA above that resulting from the primary inoculation (Fig. 2) and the plants remained symptom-free. In contrast, plants previously unexposed to W22 produced a high concentration of W22 RNA (Fig. 2) and showed disease symptoms. The resistance of recovered leaves to subsequent viral challenge suggests the existence of a resistance mechanism that restricts or prevents infection by the challenge virus.

In similar experiments, the recovered leaves of W22-infected *N. clevelandii* were inoculated with viruses that were progressively less related to W22. These analyses confirmed that the resistance associated with recovery was specific to strains that were related in genomic sequence to the recovery-inducing virus (*8*). In upper leaves challenge-inoculated with the tomato black ring nepovirus (strain BUK) there was detectable accumulation of the BUK RNA but at a substantially lower concentration in the recovered plants than in plants that were initially mock-inoculated (Fig. 2). There was also partial protection from disease induction by secondary infection with BUK (*8*). However, primary infection with W22 provided no protection against secondary infection with tomato ringspot nepovirus or with the unrelated PVX (Fig. 2).

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Of the viruses used for secondary infection, BUK is the most closely related to W22, having 68% nucleotide identity in RNA2 (9). Tomato ringspot nepovirus RNA1 and PVX RNA have no long stretches of sequence identity with W22 RNA (9). Therefore, resistance in the recovered leaves is specific for viruses that have RNA sequences that are similar to the virus used for primary inoculation.

In principle, this strain-specific resistance mechanism could be targeted against proteins encoded by the challenge viruses. Alternatively the target could be RNA, as is the case when viruses are initiators or targets of transgene silencing (2–6, 10). To distinguish between these alternative mechanisms, we tested the accumulation of a modified PVX construct (PVX.W22) in leaves of *N. clevelandii* exhibiting recovery from a primary W22 infection. PVX.W22 contained an insert of W22 sequence (Fig. 3A) at a site that did not disrupt viral replication or spread through the infected plant. The proteins required for replication and spread of PVX.W22 would be the same as those required by wild-type PVX. If the recovery was targeted against proteins, then accumulation of PVX.W22 would be unaffected by prior infection with W22. Conversely, if the recovery was targeted against the RNA of PVX.W22, then accumulation of PVX.W22 virus would be severely limited in recovered leaves of plants previously infected by W22.

Northern (RNA) analysis showed that accumulation of the PVX.W22 RNA in the recovered leaves (Fig. 3B) was below the limits of detection. In contrast, there was a high concentration of PVX.W22 RNA after inoculation to the upper leaves of mock-inoculated plants. Thus, the outcome of this experiment indicates that RNA is the target of the nepoviral recovery mechanism. The suppression of PVX.W22 in the recovered leaves was specific to the construct carrying a W22 insert, because viruses lacking sequence related to W22 accumulated to a high concentration after inoculation to both the recovered tissue and the upper leaves of the mock-inoculated plants: both wild-type PVX and PVX.GFP proliferated unhindered (Fig. 3B). The insert in PVX.GFP encodes the jellyfish green fluorescent protein (GFP) (*11*). PVX with an insert of TMV sequence was either not suppressed or was only slightly suppressed when inoculated to symptomatic systemically infected leaves of TMV-infected plants in which recovery did not occur (*12*). Thus, sequence-specific suppression of PVX constructs was characteristic of plants ex-

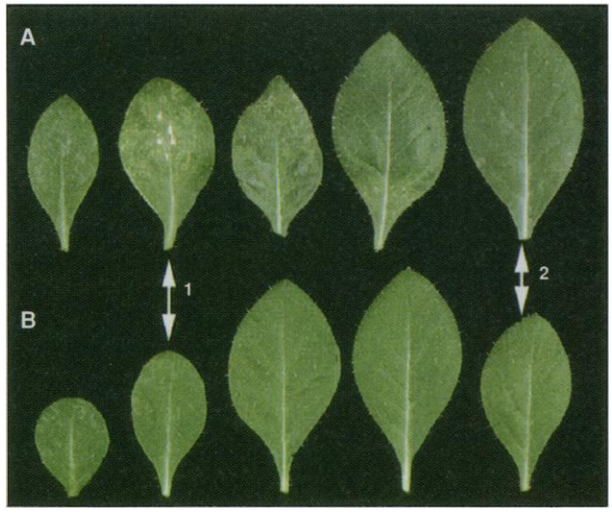

Fig. 1. Recovery in a *N. clevelandii* plant infected with tomato black ring nepovirus strain W22. A 4-week-old seedling of *N. clevelandii* was inoculated with tomato black ring nepovirus strain W22 (*16*) (**A**) or was mock-inoculated (**B**). Arrows indicate the primary (1) and secondary (2) inoculated leaves. After a further 3 weeks, the leaves were removed from the plants and are displayed from left to right in order of decreasing age on the plant.

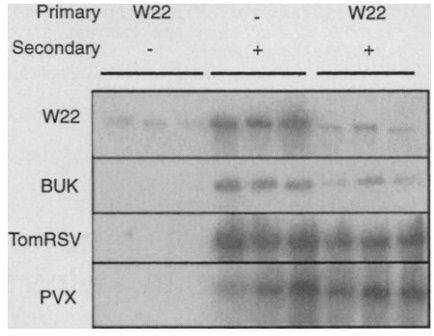

Fig. 2. Viral RNA accumulation in plants exhibiting recovery induced by nepoviral infection. Primary inoculation of 4-week-old seedlings of *N. clevelandii* was with either tomato black ring nepovirus strain W22 or water (−), as described for Fig. 1. After a further 3 weeks, an upper leaf of these plants was challenge-inoculated with water (−) or virus (+). The secondary inoculum, indicated in the left column, was tomato black ring nepovirus strain W22 (W22), tomato black ring nepovirus strain BUK (BUK), tomato ringspot nepovirus strain Wisconsin (TomRSV), or PVX (*17*). Ten days after challenge inoculation, the total RNA of the challenge-inoculated leaves was extracted (separate samples from three plants per treatment were taken). The accumulation of the challenge-inoculated RNAs was determined by Northern analysis with the use of probes specific for the challenge-inoculated virus (*18*). The figure illustrates the part of the phosphorimage of the Northern analysis showing the genomic RNAs of the challenge-inoculated viruses.

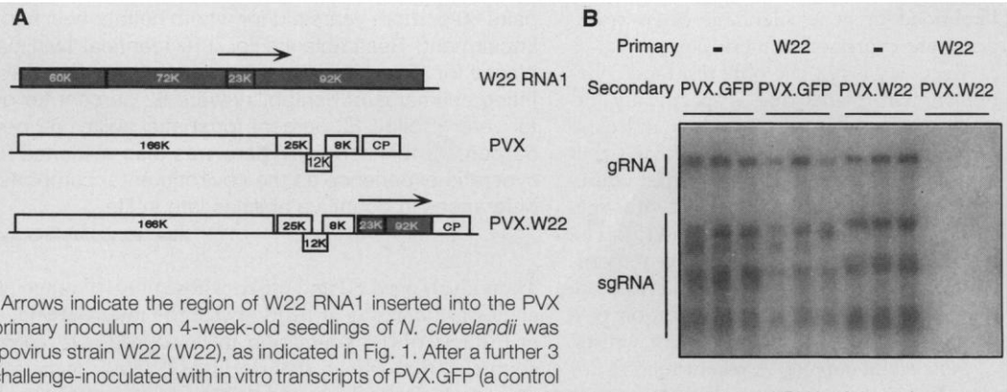

Fig. 3. Accumulation of modified PVX RNAs in plants exhibiting nepovirus-induced recovery. (**A**) Schematic diagrams of the W22 RNA1, PVX RNA, and the PVX vector construct carrying a fragment of the W22 cDNA (PVX.W22) (*19*). The diagram is not drawn to scale, but the sizes of the virus-encoded proteins are indicated (K = kilodaltons). CP is the PVX coat protein open reading frame (25 kD). W22 RNA1 has 7356 bases and PVX RNA has 6435 bases. Arrows indicate the region of W22 RNA1 inserted into the PVX vector to generate PVX.W22. (**B**) The primary inoculum on 4-week-old seedlings of *N. clevelandii* was either water (−) or tomato black ring nepovirus strain W22 (W22), as indicated in Fig. 1. After a further 3 weeks, an upper leaf of each plant was challenge-inoculated with in vitro transcripts of PVX.GFP (a control construct carrying the open reading frame for the jellyfish green fluorescent protein) or PVX.W22 (*20*). Ten days later, the total RNA of these leaves was extracted and the accumulation of the challenge-inoculated RNAs was determined by Northern analysis with the use of a PVX-specific probe (*18*). The figure illustrates a phosphorimage of the Northern analysis showing the genomic RNAs (gRNA) and subgenomic RNAs (sgRNA) of the challenge-inoculated viruses.

hibiting the nepovirus recovery phenotype and was not a general property of virus-infected tissue.

Through this analysis of nepovirus-induced recovery, we have demonstrated that a natural virus-induced effect and transgene-induced gene silencing are similar. Both phenomena are potentially virus-inducible and are associated with strain-specific virus resistance that is targeted against RNA. On the basis of these similarities, we propose that the same RNA-based mechanism underlies both phenomena. Gene silencing may occur when the plant erroneously perceives a transgene or its RNA product to be part of a virus. Transgene-induced gene silencing is normally displayed by only a small proportion of lines produced with any one construct (*6*, *13*). It may be possible to increase the incidence of gene silencing by ensuring that transgene transcripts have features, such as double-strandedness, that resemble replicative forms of viral RNA. Conversely, if it is necessary to evade gene silencing to achieve very high levels of transgene expression, it may be appropriate to produce transgenes specifying transcripts in which features resembling viral RNA are removed.

Why do nepoviruses and members of a few other virus groups elicit such pronounced recovery? One explanation, at least for nepoviruses, may follow from an earlier suggestion that there is an association between recovery and the potential of the virus to be transmitted through the seed of the infected plant (*14*). Normally, transmission through seed does not take place because viruses are excluded from the meristem and surrounding area of the plant in which gametes are produced. When seed transmission does take place, it is probably because this exclusion from the meristem has been overcome. Perhaps recovery is initiated when the nepovirus penetrates the meristem. This possible association of meristems, nepoviral recovery, and gene silencing suggests that there may be an increased likelihood of gene silencing when transgenes are expressed in meristems.

Recovery is not the only resistance phenomenon in plants that is specifically targeted against the inducing virus and close relatives. "Green islands" and mosaics that are induced by non–seed transmitted viruses are examples of localized areas of virus-specific resistance in infected plants (*15*). The relatedness of these other resistance responses and nepoviral recovery could indicate that gene silencing is a manifestation of a ubiquitous defense in plants against viruses.

Note added in proof: A recent report (*21*) also describes a recovery phenomenon in virus-infected plants that has similarity to gene silencing.

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16. The primary inoculations were to 4-week-old seedlings maintained in a glasshouse under added artificial illumination at a temperature between 20° and 25°C.
17. Cultures of tomato black ring nepovirus and of tomato ringspot nepovirus (Wisconsin strain) were obtained from the Scottish Crop Research Institute, Dundee, UK. They were propagated by inoculation to *Nicotiana* sp. The crude sap of infected plants was used as inoculum for these viruses. PVX inocula consisted of sap of plants infected with in vitro transcripts of a full-length cDNA clone (pTXS) (*11*).
18. The probe for PVX was ^{32}P-labeled cDNA from the 3′ terminal 1.5 kb of the PVX genome. For tomato black ring nepovirus strains W22 and BUK, the probes were ^{32}P-labeled polymerase chain reaction (PCR)–amplified cDNAs. The primers for PCR amplification of these probes were from nucleotides 1470 to 1498 and 2625 to 2650 of the RNA2 of W22 that are conserved in both strains (*9*). The probe for tomato ringspot nepovirus was ^{32}P-labeled cDNA produced by oligo dT–primed reverse transcription of purified tomato ringspot viral RNA. RNA isolation and Northern analysis were done as described previously (*11*). The Northern analysis images were generated on a Fuji BAS1000 phosphorimager.
19. The construct pPVX.W22 was obtained by transfer of a cDNA fragment of W22 into the Eco RV site of PVX vector cDNA in pP2C2S (*11*). This cDNA fragment was obtained by PCR amplification of W22 RNA1 between nucleotide positions 4431 and 5409 of the sequence of RNA1 (*9*).
20. PVX.GFP and PVX.W22 were inoculated directly as transcripts of the cDNA clones pTXS.GFP (*11*) or pPVX.W22. Each plant was inoculated with the equivalent of a 5-μl transcription reaction. The probes for PVX.W22 and PVX.GFP were ^{32}P-labeled cDNA from the 3′ terminal 1.5 kb of the PVX genome.
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Substantial Genetic Influence on Cognitive Abilities in Twins 80 or More Years Old

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General and specific cognitive abilities were studied in intact Swedish same-sex twin pairs 80 or more years old for whom neither twin had major cognitive, sensory, or motor impairment. Resemblance for 110 identical twin pairs significantly exceeded resemblance for 130 fraternal same-sex twin pairs for all abilities. Maximum-likelihood model-fitting estimates of heritability were 62 percent for general cognitive ability, 55 percent for verbal ability, 32 percent for spatial ability, 62 percent for speed of processing, and 52 percent for memory. There was also evidence for the significant influence of idiosyncratic experience as the environmental component that most determines individual differences in cognitive abilities late in life.

Individuals aged 80 and older, whose prevalence is increasing at nearly twice the rate of the rest of the population in developed countries throughout the world (*1*), vary immensely in health and functional capabilities. Little is known about the genetic and environmental origins of this wide range of individuality (*2*–*4*). A particularly crucial aspect of quality of life in the elderly is cognitive functioning, which includes general and specific cognitive abilities. General cognitive ability, which represents that which diverse cognitive abilities have in common, is frequently measured by a