αβ subunit interfaces are extensive, include the P cluster in both Δ*nifB*-Av1 and Av1, and should remain intact. However, the rearrangement of the αIII domain, in addition to generating the opening for FeMoco insertion, may also create a potential interaction domain that enables residues found in the disordered region of the Δ*nifB*-Av1 structure to dock with the FeMoco donor.

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Outbreak of Poliomyelitis in Hispaniola Associated with Circulating Type 1 Vaccine-Derived Poliovirus

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An outbreak of paralytic poliomyelitis occurred in the Dominican Republic (13 confirmed cases) and Haiti (8 confirmed cases, including 2 fatal cases) during 2000–2001. All but one of the patients were either unvaccinated or incompletely vaccinated children, and cases occurred in communities with very low (7 to 40%) rates of coverage with oral poliovirus vaccine (OPV). The outbreak was associated with the circulation of a derivative of the type 1 OPV strain, probably originating from a single OPV dose given in 1998–1999. The vaccine-derived poliovirus associated with the outbreak had biological properties indistinguishable from those of wild poliovirus.

The last case of poliomyelitis in the Americas associated with circulating indigenous wild poliovirus was reported in Peru in 1991 (*1*). The last poliomyelitis cases in the Caribbean occurred on the island of Hispaniola, which is divided between the Dominican Republic (where the last reported case was in 1985) and Haiti (where the last reported case was in 1989). Since 1992, investigation of nearly 20,000 cases of acute flaccid paralysis (AFP) throughout the Americas (*2*) has found only sporadic cases of vaccine-associated paralytic poliomyelitis (VAPP) (*3*). The only known wild poliovirus infections in the Americas after 1991 were associated with importations into Canada (*4*, *5*). Neither of these importations was associated with paralytic disease, and there was no evidence of the spread of the virus to the wider community.

In the summer of 2000, cases of AFP were reported from Bonao, Dominican Republic (date of onset: 12 July), and Port-de-Paix, Haiti (date of onset: 28 August), communities that are separated by ~300 km (Fig. 1) (*6*). Stool specimens collected from the patients were positive for poliovirus type 1. An initial characterization of the isolates by nucleic acid probe hybridization (*7*) indicated that they were vaccine-derived poliovirus (VDPV). In countries using oral poliovirus vaccine (OPV), VDPV is occasionally isolated from the stools of patients with nonpolio AFP (*2*, *3*). However, several observations raised concerns that the AFP cases in Hispaniola might be caused by

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circulating wild poliovirus: (i) both patients had residual paralysis, (ii) the cases occurred during the peak season for enterovirus (and poliovirus) transmission, (iii) one patient was unimmunized and the other was incompletely immunized (had been given one OPV dose), and (iv) type 1 poliovirus is the primary agent of poliomyelitis outbreaks (*8*). A sequence characterization (*6*) of the two isolates, comparing the major capsid surface protein VP1, showed that they were unrelated [<82% VP1 sequence identity (*6*)] to wild type 1 polioviruses previously endemic to Hispaniola or elsewhere in the Americas or to any wild poliovirus currently found in other parts of the world (*9*) (Fig. 2). By contrast, the Haitian and Dominican isolates were closely related (97.4 and 98.1% VP1 sequence identity, respectively) to the Sabin type 1 OPV strain and to each other (98.0% VP1 sequence identity). However, the degree of VP1 sequence similarity to the OPV strain was substantially lower than is normally observed (>99.5%) in isolates from cases of AFP or VAPP. Unlike the two cases in Hispaniola, cases of VAPP are rare (~1 case per 2 million doses of OPV distributed), sporadic, involve independent exposures to OPV, and are most frequently associated with poliovirus types 2 and 3 (*10*). Moreover, the sequence relationships between the two isolates (12 of the VP1 nucleotide substitutions distinguishing the isolates from Sabin 1 were shared between them) suggested that they were derived from a recent common ancestor, as is typical of wild polioviruses isolated during an outbreak (*11*, *12*). Consequently, AFP surveillance was intensified to search for additional polio cases.

From 12 July 2000 to 31 July 2001, a total of 123 AFP cases were reported in the Dominican Republic, 13 of which have been confirmed as polio by isolation of poliovirus type 1 from either the patients or their healthy contacts (Fig. 1) (*6*). In Haiti, 33 AFP cases were investigated between 28 August 2000 and 31 July 2001. Seven additional cases associated with circulating type 1 VDPV were found in 2001 (Fig. 1). Two patients died of bulbar poliomyelitis. Only one patient had a record of receiving three doses of OPV. All others were unvaccinated (11 patients), incompletely vaccinated (7 patients), or had an unknown vaccination status (2 patients) (*6*).

Type 1 VDPV was isolated from 19 polio patients and 12 healthy contacts in the two countries (*6*). All 31 isolates were closely related to each other and appeared to be the progeny of a recent common OPV infection (Fig. 3). The VP1 nucleotide diversity (*6*) of the Haitian isolates (0.030 nucleotide differences per site) was substantially higher than that of the Dominican isolates (0.007 nucleotide differences per site). Isolates from the north of Haiti were more closely related to each other than they were to the isolates from the south, which in turn represented three distinct lineages (Fig. 3).

The approximate times of key events in the transmission history of the outbreak virus can be estimated from the VP1 sequence relationships among isolates, the dates of specimen collection, and the rate of VP1 evolution (*11*). By assuming that the evolution rate within VP1 of the outbreak virus was 0.030 substitutions per synonymous site per year (*6*, *11*), we estimated that the initiating OPV dose occurred in late 1998 or early 1999. We further estimated that the northern and southern lineages in Haiti diverged in mid-1999 and that the southern lineages diverged again a few months later. The close sequence relationships among the Dominican isolates suggested that the common ancestral infection for this clade occurred in the spring of 2000.

All outbreak virus isolates were recombinants. The 5′–untranslated and capsid-region sequences were derived from Sabin 1, whereas most of the noncapsid sequences were derived from other species C enteroviruses (Fig. 4) (*13*). A block of recombinant sequences (positions 3811 to ~3952; $2A^{pro}$/2B region) was shared by all outbreak isolates. The close relationship (93.7 to 100% se-

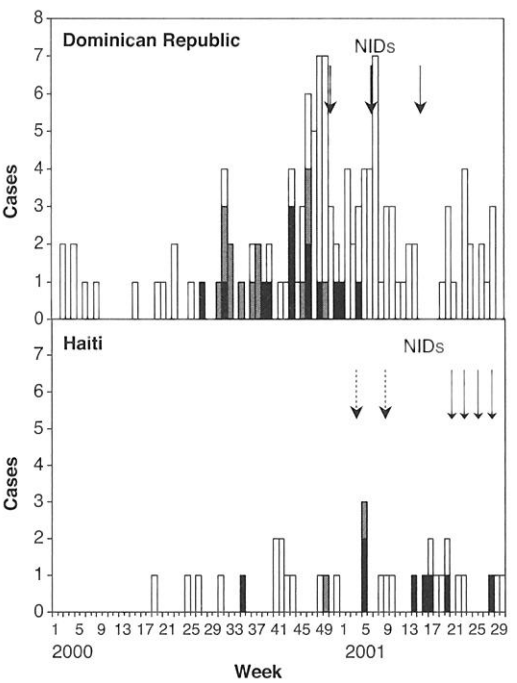

Fig. 1. Temporal distribution of confirmed cases of paralytic poliomyelitis (black bars), polio-compatible acute flaccid paralysis (AFP) cases (gray bars), and other AFP cases (white bars) in the Dominican Republic (upper panel) and Haiti (lower panel) from 1 January 2000 to 31 July 2001. Confirmed cases had type 1 VDPV isolated from stool. Polio-compatible cases had residual paralysis at 60 days after onset but no isolation of type 1 VDPV. Arrows indicate the dates of NIDs. The dashed arrow shafts indicate suboptimal OPV coverage of the first two NIDs in Haiti; the row of smaller arrows symbolizes the May to July 2001 rolling immunization campaigns in Haiti.

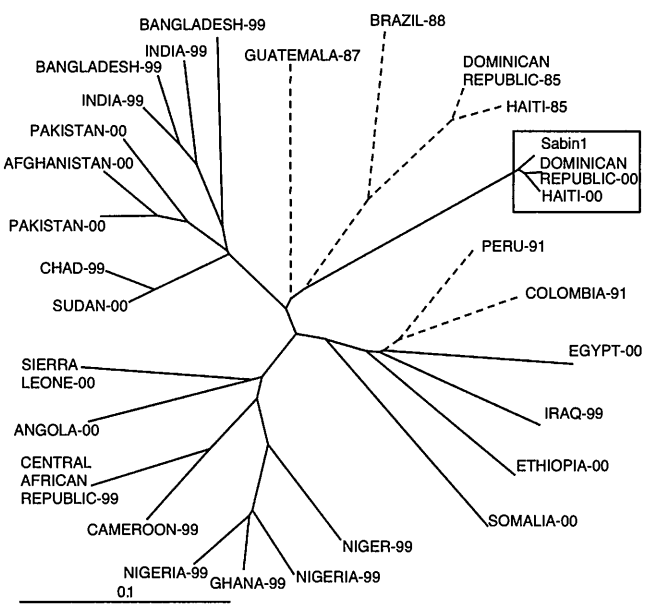

Fig. 2. Unrooted neighbor-joining tree (*6*) summarizing VP1 (nucleotides 2480 to 3385) sequence relationships among the Sabin 1 OPV strain, the first isolates from the 2000 outbreak in the Dominican Republic (case DOR00-013) and Haiti (case HAI00-003), the last isolates of the wild type 1 polioviruses previously indigenous to the Dominican Republic (1985) and Haiti (1985), the last isolates of the other type 1 poliovirus genotypes previously indigenous to the Americas (dashed lines connect to extinct genotypes), and 21 wild type 1 poliovirus isolates from cases occurring in polio-endemic countries of Africa and Asia in 1999 and 2000.

quence identity) among sequences within this block provides strong evidence of their introduction by a single recombination event and confirms that the outbreak virus was derived from a single source infection. Noncapsid sequences of the 21 Dominican isolates were generally very similar, except for isolates from three contacts of a Navarrete case (DOR00-041) (Figs. 3 and 4) (*6*), which had $3D^{pol}$ region sequences derived from another enterovirus. Noncapsid sequence relationships among the 10 Haitian isolates were more complex. Seven southern isolates and the Pilate isolate had recombination breakpoints in common with the main Dominican group. However, the isolates from the Port-de-Paix case and the fatal Port-au-Prince case differed in their noncapsid sequences from the other Haitian and Dominican isolates and from each other (Fig. 4). At least four different enteroviruses recombined with the type 1 VDPV during its circulation in Hispaniola.

The outbreak VDPV had recovered two of the most important biological properties of wild polioviruses: the capacity to cause severe paralytic disease in humans and the capacity for extensive person-to-person transmission. All 31 isolates had a G→A substitution at nucleotide 480 in the 5′-untranslated region. This substitution restored a 480A:525U base pair within a dual stem-loop secondary structural element of the internal ribosome entry site that controls the efficiency of translation of poliovirus RNA (*14*) and represents a reversion of an important determinant of the attenuated phenotype of the Sabin 1 OPV strain (*15–17*). Other alleles that confer attenuation or temperature sensitivity to Sabin 1 (*16*) had either reverted (at positions 935, 2438, and 2795) or had been exchanged out by recombination (at positions 6143, 6203, 7071, and 7441) (*6*). The genetic properties of the outbreak isolates were consistent with their association with paralytic disease in humans and with other biological properties determined experimentally. The first two outbreak isolates were found to be highly neurovirulent for PVR–Tg21 transgenic mice expressing the human receptor for poliovirus (*6*), and unlike Sabin 1, could replicate to high titers in HeLa cells at 39.5°C (*6*). Amino acid substitutions in virion surface residues (*6*, *18*) correlated with the "non-vaccinelike" antigenic properties of the VDPV isolates (*19*).

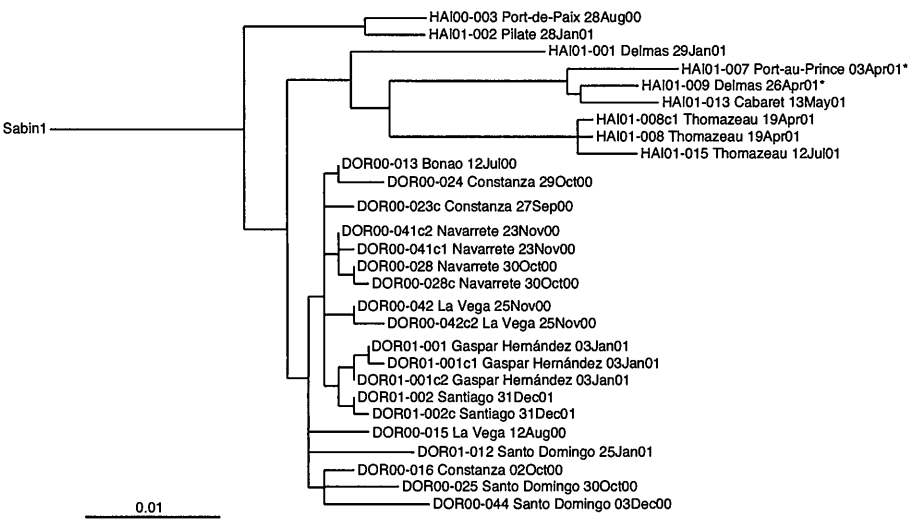

Fig. 3. Maximum likelihood tree (*6*) of VP1 sequence relationships among the Sabin 1 OPV strain (root of tree) and 28 type 1 VDPV isolates from the 2000–2001 outbreak in the Dominican Republic and Haiti. The location of the case and date of onset of paralysis are shown for each isolate. The isolates from the fatal cases in Haiti (HAI01-007 and HAI01-009) are indicated by asterisks. Isolate pairs that had identical VP1 sequences (DOR00-041c2, DOR00-041c3; DOR00-042, DOR00-042c1; HAI01-008c1, HAI01-008c2) are represented only once on the tree.

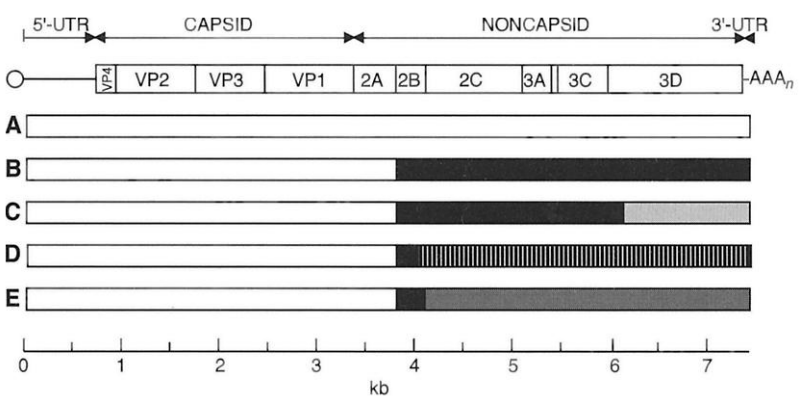

Fig. 4. Location of recombination breakpoints of the four different recombinant classes found among Hispaniola VDPV isolates. In the schematic of the poliovirus genome, the single open reading frame is indicated by a rectangle, flanked by the 5′- and 3′-untranslated regions (UTR). In rectangles A to E, Sabin 1–derived sequences are indicated by white fill, and sequences derived from enteroviruses other than the Sabin OPV strains are indicated by shaded or hatched fills. The rectangles symbolize sequences of A, Sabin 1; B, DOR00-013; C, DOR00-041c1; D, HAI00-003; and E, HAI01-007 (*6*).

Circulation of VDPV occurred in an environment of low vaccine coverage throughout Haiti and in parts of the Dominican Republic. In Haiti, reported coverage rates for three doses of OPV (OPV3) for children <1 year of age were the lowest in the Americas, falling to around 30% nationwide from 1992 to 1997, and remaining below 60% by 2000. Nationwide OPV3 coverage rates were higher in the Dominican Republic (ranging from 72 to 84% from 1993 to 2000), but coverage was very low (20 to 30%) in the outbreak communities. No mass OPV immunization campaigns in the form of National Immunization Days (NIDs) had been conducted in either country within the last 5 years.

The gaps in vaccine coverage were accompanied by gaps in surveillance, especially in Haiti. Nonetheless, a reasonable reconstruction of the events leading to the outbreak can be inferred from the combined epidemiologic and virologic data. The outbreak probably began in Haiti, when a routine OPV dose was given to a child living in a community with low vaccine coverage. As VDPV excreted by the OPV recipient spread to nonimmune children in the community, variants may have been selected for increased neurovirulence (*14*, *20*) and transmissibility potentials. The outbreak in the Dominican Republic began with the importation of virus from one of the Haitian VDPV lineages into a low–OPV coverage community.

Evidence of circulating VDPV has recently been found elsewhere. In the Philippines, circulating type 1 VDPV has been associated with three cases of AFP in 2001 (*21*). In Egypt, type 2 VDPV circulated for an estimated 10 years (from 1983 to 1993) and was associated with 32 reported cases (*22*). In these examples, as in Hispaniola, the critical risk factors were poor population immunity resulting from gaps in OPV coverage and the absence of indigenous circulation of the corresponding wild poliovirus serotype.

The finding that VDPV can, under suit-

able conditions, circulate and cause cases of poliomyelitis has important implications for current and future strategies of the World Health Organization (WHO) initiative to eradicate polio worldwide (*23*). First, the eradication of wild poliovirus, now at an advanced stage (*23*), must be completed as soon as possible. At the same time, it is imperative that immunity gaps in nonendemic countries are prevented, especially in tropical developing countries where the risk for poliovirus circulation is highest (*24*). After certification of wild poliovirus eradication, a carefully planned strategy for the orderly cessation of OPV use worldwide should be implemented. Finally, sensitive global poliovirus surveillance must be maintained for the foreseeable future, and emergency stockpiles of poliovirus vaccine established, for use in the event of any recurrent poliovirus transmission from chronic poliovirus excretors (*25*), a breach in poliovirus containment (*25*), or circulating VDPV.

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Requirement for a Peptidoglycan Recognition Protein (PGRP) in Relish Activation and Antibacterial Immune Responses in *Drosophila*

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Components of microbial cell walls are potent activators of innate immune responses in animals. For example, the mammalian TLR4 signaling pathway is activated by bacterial lipopolysaccharide and is required for resistance to infection by Gram-negative bacteria. Other components of microbial surfaces, such as peptidoglycan, are also potent activators of innate immune responses, but less is known about how those components activate host defense. Here we show that a peptidoglycan recognition protein, PGRP-LC, is absolutely required for the induction of antibacterial peptide genes in response to infection in *Drosophila* and acts by controlling activation of the NF-κB family transcription factor Relish.

In response to infection, *Drosophila* activates the transcription of a battery of antimicrobial peptide genes in cells of the fat body (the insect analog of the liver). Two major branches of this humoral response have been identified; as in mammals, these responses require NF-κB transcription factors (*1*). One branch activates antifungal responses and requires the receptor Toll and the NF-κB family transcription factor Dif (*2–4*). The second branch, which is primarily antibacterial, requires the NF-κB protein Relish, an IκB kinase (IKK), a caspase, a mitogen-activated protein kinase kinase kinase, and the death-domain protein Imd (*5–11*).

We have taken a genetic approach to identifying genes required for the antibacterial response (*12, 13*). One gene that is absolutely required for the induction of the antibacterial response is *ird7* (*immune response deficient 7*). Two mutations in *ird7* identified in an ethylmethane sulfonate (EMS) mutagenesis screen (*12, 13*) prevented the induction of three antibacterial peptide genes, *Diptericin*, *Cecropin*, and *Defensin*, after infection by either Gram-negative or Gram-positive bacteria (Fig. 1, A and B). Three other antimicrobial peptide genes, *Attacin*, *Metchnikowin*, and *Drosomycin*, also failed to be induced to normal levels. The profile of antimicrobial gene expression observed in the *ird7* mutants was similar to that observed in *imd*, *DmIkkβ/ird5*, and *Relish* mutants after bacterial infection, but was distinct from that of *Toll* and *Dif* mutants (Fig. 1A). This pattern suggests that *ird7* is an essential component of the same signaling pathway that requires *imd* and *Relish*, but is not required for the Toll-Dif pathway. Both *ird7* mutants are homozygous viable and fertile, and blood cells from *ird7* mutants can phagocytose bacteria (*14*); these findings suggest that *ird7* is required specifically for the humoral immune response.

The transcription factor Relish directly activates antibacterial target genes in *Drosophila*. Relish is a compound protein similar to mammalian p100 and p105 (the precursors of the p52 and p50 subunits of NF-κB), with an NH_2-terminal Rel homology and a COOH-terminal ankyrin repeat domain similar to that of the NF-κB inhibitor IκB (*15*). In response to immune challenge, full-length Relish (REL-110) is endoproteolytically clipped to generate the NH_2-terminal REL-68 fragment, which translocates into the nucleus, and the COOH-terminal REL-49 ankyrin repeat fragment, which remains stable in the cytoplasm (*16*) (Fig. 1C). In contrast to wild-type animals, no processing of Relish was detected in *ird7* mutant larvae (Fig. 1C). The

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