calculate that the oscillation period is $T = 1/f_m = 100$ ps, which matches the time delay between relaxation peaks in Fig. 5B. The transform-limited linewidth resulting from 40-ps pulses is $\Delta\lambda = 0.2 \pm 0.1$ Å, which matches the measured spectral linewidth of the OVCSELs.

The ultimate limit for the linewidth can be calculated from conventional semiconductor laser theory through the use of (*16*):

$$P\Delta\nu = \frac{h\nu g_{TH} n_{sp} \nu_{g0}^2 \xi^2}{16\pi d} \left[\frac{(\sqrt{R_1} + \sqrt{R_2})(1 - \sqrt{R_1 R_2})(1 - R_1)}{R_1 \sqrt{R_2} \ln(1/\sqrt{R_1 R_2})} \right] (1 + \alpha^2) \quad (1)$$

Here $\nu_{g0} = c/n$, where c is the speed of light in vacuum. The Bragg reflector and buffer layer act as the external cavity of total length $L = (5/2)\,\lambda/n \approx 930$ nm for $\lambda = 635$ nm, and the fraction of the mode in the active region is approximately $\xi = 0.5$. Also, $\alpha = \Delta n/\Delta n'$ is the linewidth enhancement factor, where Δn and $\Delta n'$ correspond to changes in the real and imaginary parts of the refractive index, respectively, due to the change in carrier density upon optical excitation. Finally, $n_{sp} = N_1/(N_1 - N_0)$, where N_1 and N_0 are the populations of the excited and ground states, respectively.

The linewidth of OSLs is comparable to that of inorganic semiconductor lasers with similar mirror reflectivities. A guest-host molecular system such as Alq_3:DCM can be treated as a four-level system, for which $n_{sp} = 1$ because the lower state involved in the radiative transition is unoccupied ($N_0 = 0$), whereas in inorganic semiconductors n_{sp} is typically near 2 or more (*16*). The main reduction in the linewidth of OSLs, however, is related to the α-parameter, which is well below 1 over a wide spectral range for materials such as Alq_3:DCM, whereas α ranges from 2 to 5 for most inorganic semiconductor lasers (*16*). We have estimated α (Fig. 4, right inset) by calculating $\Delta n'$ from the spontaneous emission spectrum using the Einstein relation (*17*), and Δn from $\Delta n'$ using the Kramers-Kronig relation. Because the spontaneous emission spectrum is broad and almost symmetric, α is small in the wavelength region centered near the emission peak, with $\alpha = 0.2$ at $\lambda = 635$ nm. In comparison to inorganic semiconductor lasers, OSLs have a larger ν_{g0} due to lower n, a larger g_{TH}, as well as a shorter d, all of which contribute to an increase in $P\Delta\nu$, thus partially offsetting linewidth-narrowing effects due to a small α. The power-linewidth product from Eq. 1 is then $P\Delta\nu = 1$ GHz mW, comparable to that of inorganic semiconductor lasers. However, the small Δn of OSLs implies a smaller chirp (narrower pulse width) as compared to inorganic semiconductor lasers.

Optical gain in vacuum-deposited organic thin films is sufficient for obtaining very high output power, optically pumped vertical-cavity surface-emitting lasers. Using these structures in an electrically pumped configuration will require reducing the lasing threshold of 300 μJ/cm^2 to values near 0.1 μJ/cm^2, assuming a pulsed peak pump current of ~1 kA/cm^2. The use of a higher top contact reflectivity or more optically efficient organic systems (*5*) may help realize this goal.

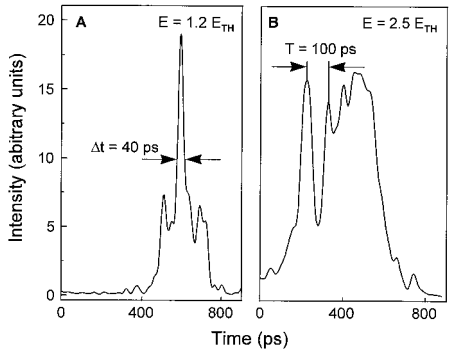

Fig. 5. Normalized temporal behavior of OVSCEL emission at excitation levels of (**A**) $E = 1.2\,E_{TH}$ and (**B**) $E = 2.5\,E_{TH}$, indicating the presence of relaxation oscillations of 40-ps duration at 100-ps intervals.

REFERENCES AND NOTES

1. V. G. Kozlov, V. Bulović, P. E. Burrows, S. R. Forrest, *Nature* **389**, 362 (1997).
2. V. G. Kozlov, V. Bulović, S. R. Forrest, *Appl. Phys. Lett.* **71**, 2575 (1997).
3. N. Tessler, G. J. Denton, R. H. Friend, *Nature* **382**, 695 (1996).
4. M. A. Díaz-García *et al*., *Appl. Phys. Lett.* **70**, 3191 (1997).
5. M. Berggren, A. Dodabalapur, R. E. Slusher, Z. Bao, *Nature* **389**, 466 (1997).
6. S. R. Forrest, P. E. Burrows, E. I. Haskal, F. F. So, *Phys. Rev.* ***B*** **49**, 11309 (1994); S. R. Forrest, *Chem. Rev.* **97**, 1793 (1997).
7. V. G. Kozlov, G. Parthasarathy, P. E. Burrows, S. R. Forrest, *Appl. Phys. Lett.* **72** (12 January 1998), p. 144.
8. P. E. Burrows *et al*., *J. Appl. Phys.* **79**, 7991 (1996).
9. G. J. Denton, N. Tessler, M. A. Stevens , R. H. Friend, *Adv. Mater.* **9**, 547 (1997).
10. L. A. Coldren and S. W. Corzine, *Diode Lasers and Photonic Integrated Circuits* (Wiley, New York, 1995).
11. M. Pope and C. E. Swenberg, *Electronic Processes in Organic Crystals* (Oxford Univ. Press, Oxford, UK, 1982).
12. F. P. Schafer, *Dye Lasers* (Springer-Verlag, Berlin, 1977).
13. The OVCSEL and pump laser power was measured with a large-area detector connected to Molectron EPM 1000 energy/power meter.
14. A. Costela, I. Garcia-Moreno, J. M. Figuera, F. Amat-Guerri, R. Sastre, *Appl. Phys. Lett.* **68**, 593 (1996).
15. Finesse is defined as the ratio of microcavity-mode spacing to a single-mode linewidth.
16. C. H. Henry, *J. Lightwave Technol.* ***LT*****-4**, 288 (1986).
17. See, for example, M. Weissbluth, *Atoms and Molecules* (Academic Press, New York, 1978).

18. We are grateful to the Air Force Office of Scientific Research (C. Lee and M. Prairie), the National Science Foundation Materials Research Science and Engineering Center, and the Universal Display Corporation for support of this work.

14 October 1997; accepted 5 December 1997

Accelerating Invasion Rate in a Highly Invaded Estuary

Andrew N. Cohen* and James T. Carlton

Biological invasions are a major global environmental and economic problem. Analysis of the San Francisco Bay and Delta ecosystem revealed a large number of exotic species that dominate many habitats in terms of number of species, number of individuals and biomass, and a high and accelerating rate of invasion. These factors suggest that this may be the most invaded estuary in the world. Possible causes include a large number and variety of transport vectors, a depauperate native biota, and extensive natural and anthropogenic disturbance.

Over the past few centuries, thousands of species of freshwater, estuarine, and marine organisms have dispersed outward from their native regions through human-mediated transport and have established sustaining populations in distant parts of the globe (*1*, *2*). Many of these organisms have profoundly affected the abundance and diversity of native biota in the regions they have invaded (*3*, *4*), and in some cases they have had substantial economic impacts (*5*). Despite these many invasions, data sets suitable for analysis of spatial or temporal patterns of aquatic invasions are rare. Here, we analyzed a synthesis of such data for one of

A. N. Cohen, San Francisco Estuary Institute, 1325 South 46 Street, Richmond, CA 94804, USA.
J. T. Carlton, Maritime Studies Program, Williams College–Mystic Seaport, Post Office Box 6000, Mystic, CT 06355, USA.

*To whom correspondence should be addressed. E-mail: acohen@sfei.org

the largest estuarine and freshwater ecosystems in North America, the San Francisco Bay and Delta. This ecosystem comprises 1500 km^2 of aquatic habitat within the range of normal tidal influence. It receives runoff from a 163,000-km^2 watershed with an extensive system of dams, water diversions, and flood channels that partially control river flows, which nevertheless exhibit considerable seasonal and annual variation. Water salinities also vary widely, generally ranging from fresh in the Delta to coastal salinities near the mouth of the Bay, and sometimes hypersaline conditions during droughts in southern parts of the Bay (*6*).

We assembled data on introduced aquatic organisms from published and unpublished data, augmented by our field work. Exotic species were defined as those not present in the eastern North Pacific bioregion before the entry of Europeans in the 16th century, or present in distant parts of that region and later introduced to the Bay/Delta ecosystem by human-mediated mechanisms (*7*). Cryptogenic species are species that are neither clearly native nor exotic (*8*). In earlier reports we described the evidence used to distinguish between native and exotic species and to determine establishment (*9*).

We identified a total of 234 exotic species established in the ecosystem, including plants, protists, invertebrates, and vertebrates (Table 1). At least 125 additional species are cryptogenic. We sorted species according to their native regions, counting purely freshwater organisms as continental organisms and the rest as marine. Most continental organisms derived from eastern North America and Europe, most marine organisms from the North Atlantic and the western North Pacific (Table 2). A review of sampling data and species lists revealed that exotic species dominate many of the ecosystem's biotic associations, including infaunal and epifaunal soft-bottom benthos (organisms living within or on the bottom sediments), fouling communities, brackish-water zooplankton, and freshwater fish. In these communities, exotic organisms typically account for 40 to 100% of the common species, up to 97% of the total number of organisms, and up to 99% of the biomass (*10*).

In our analysis of changes in the rate of invasion, we used raw data as well as a data set modified by the exclusion of each record for which we could not determine the year of planting, observation, or collection, or that we judged to result from special expertise, an extraordinary collection effort, or the chance discovery of a highly localized species. The modified data set excluded about one-third of the raw data. The shapes of the cumulative invasions curves (Fig. 1) are similar for the raw and modified data. The raw data show that 55.2% of the total number of invasions were recorded after 1960, versus 49.7% for the modified data set. Thus, about half of all invasions in the 145-year record were reported in the last 35 years. On the basis of the raw data, the rate of invasions has increased from an average of one new species established every 55 weeks from 1851 to 1960, to an average of one new species every 14 weeks from 1961 to 1995.

Taken together, the large number of exotic species, their dominance in many habitats, and the rapid and accelerating rate of invasion suggest that the San Francisco Bay and Delta may be the most invaded estuary and possibly the most invaded aquatic ecosystem in the world. Although some other aquatic systems are known to host many exotic species (*3*, *11*), few report as many and none report the extensive dominance by exotic organisms that is shown here (*12*). Several factors could be contributing to the extent of invasion in this system:

1) Transport vectors. The Bay/Delta system may have been inoculated by a greater number or diversity of potential invaders than have other estuaries. Many mechanisms, connecting at different times to different regions of the world and favoring different components of the biota, have transported organisms into this ecosystem, including transport with ships; transport with oysters imported for cultivation; fish stocking; releases or escapes from commercial and government breeding and rearing facilities, ornamental ponds, and aquariums; introductions for biological control; plantings of exotic vegetation for marsh "restoration" and erosion control; and importation with shipments of live seafood or bait. The scale and diversity of such mechanisms may be increasing with the expansion in international commerce.

2) Depauperate biota. It has been argued that the relative youth of northeast Pacific estuaries, or their island-like isolation, prevented the development of a species-rich native biota that could resist invasions (*13*). However, theories that island, young, or

Table 1. Exotic species established in the San Francisco Bay and Delta. Organisms that reproduce in both fresh and salt or brackish waters, or that move between them as a regular part of their life cycle (anadromous and catadromous species), were counted in both environments. Other organisms were counted in the environment in which they reproduce.

Taxonomic group	Number of species in salt or brackish water	Number of species in fresh water	Total number of species
Seaweeds	6	0	6
Vascular plants	12	18	25
Protozoans	8	0	8
Sponges	5	0	5
Cnidarians	16	1	17
Flatworms	0	9	9
Nematodes	0	1	1
Annelids	15	8	21
Mollusks	27	3	30
Arthropods	51	11	60
Entoprocts	1	1	2
Bryozoans	9	0	9
Tunicates	8	0	8
Fish	6	29	30
Amphibians	0	1	1
Reptiles	0	1	1
Mammals	0	1	1
Total	164	84	234

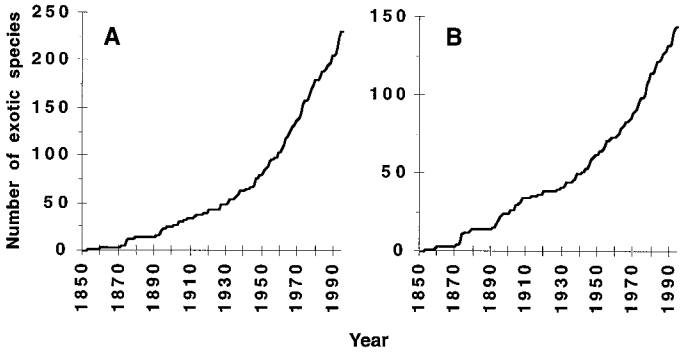

Fig. 1. Cumulative number of exotic species established in the San Francisco Estuary: (**A**) raw data; (**B**) modified data. A time series analysis conducted by C. Parmesan [Box-Jenkins methodology (*19*) on RATS (Regression Analysis of Time Series) software, version 4.0 (Estima); final models used only terms that contributed significantly at $P < 0.05$ and had uncorrelated residuals by Durban-Watson and Lung-Box Q statistics] showed a significant increase in the number of invasions over time (linear regression on raw and modified data separately, trend terms significant at $P < 0.001$). Trend = (time)^2 models explained 5% and 2% more of the variation than did trend = time models for raw and modified data sets, respectively (final models: $R^2_{raw} = 0.34$, $R^2_{modified} = 0.21$). Additional lag terms on time were unnecessary (and when forced into the models were not significant at $P > 0.50$ for both data sets), indicating that invasion events are independent between years. A better fit of trend = (time)^2 models indicates that the rate of invasions, as well as the absolute number, increases with time.

species-poor communities are more vulnerable to invasion than are other communities (*1*, *14*) have been tested only occasionally and have been challenged (*15*). Nor can such theories explain the observed increase in invasion rate as more species became established.

3) Disturbance. It has been argued both that extreme natural disturbance events facilitated the establishment of some exotic organisms in the Bay/Delta system (*16*) and that human alterations of habitat have made the system vulnerable to invasions (*17*), consistent with the view that disturbed environments are more easily invaded (*1*, *18*). Although greater numbers or greater dominance of exotic species in disturbed areas relative to undisturbed areas has often been observed, it is unclear whether this occurs because these areas are more easily invaded, because they are more heavily inoculated with exotic organisms, or because a greater number of the inoculated organisms are adapted to the disturbed environments from which they were imported. It is also unclear whether the Bay/Delta system is any more disturbed than other estuaries.

The relative contribution of these factors to the extent of invasion might be illuminated by comparative studies with other estuaries, but these must await the development of comprehensive regional data sets on invasions similar to the one discussed here. Proceeding with such work would be of great value. Our study shows the San Francisco Bay and Delta to be extensively invaded, seemingly far more so than other large aquatic ecosystems. Given the potential impact of such invasions on both native biological diversity and human economic activities, it is a matter of some urgency to learn why.

Table 2. Native regions of exotic species that have become established in the San Francisco Bay and Delta. Where native regions are imprecisely known (for example, the organism could be native to either the eastern or western North Atlantic), the count was split, so figures for some regions include "half" species.

Source regions	Number of species
Marine	
Western North Atlantic	52.5
Western North Pacific	41.5
Eastern North Atlantic	20.5
Western South Pacific	8
Mediterranean, Black, and Caspian seas	5
Indian Ocean	3.5
Eastern South Pacific	2
Unknown	25
Continental	
North America	39.5
Eurasia	25.5
South America	7
Africa	2
Unknown	2
Total	234

REFERENCES AND NOTES

1. C. S. Elton, *The Ecology of Invasions by Animals and Plants* (Methuen, London, 1958).
2. L. Walford and R. Wicklund, *FAO Fisheries Tech. Pap. 121* (1973); R. L. Welcomme, *FAO Fisheries Tech. Pap. 213* (1981); W. R. Courtenay Jr., D. A. Hensley, J. N. Taylor, J. A. McCann, in *The Zoogeography of North American Fishes*, C. H. Hocutt and E. O. Wiley, Eds. (Wiley, New York, 1986), pp. 675–698; J. T. Carlton, *Conserv. Biol.* **3**, 265 (1989).
3. H. Zibrowius, *Mésogée* **51**, 83 (1991).
4. E. Leppäkoski, in *Nonindigenous Estuarine and Marine Organisms (NEMO)* (National Oceanic and Atmospheric Administration, Washington, DC, 1993), pp. 37–44; M. A. Ribera and C. F. Boudouresque, in *Progress in Phycological Research*, F. E. Round and D. J. Chapman, Eds. (Biopress, Amsterdam, 1995), vol. 11, pp. 187–268.
5. T. F. Nalepa and D. W. Schloesser, Eds., *Zebra Mussels: Biology, Impacts, and Controls* (Lewis, Boca Raton, FL, 1993); J. Travis, *Science* **262**, 1366 (1993); *Harmful Non-indigenous Species in the United States* (U.S. Congress, Office of Technology Assessment, Washington, DC, 1993).
6. T. J. Conomos, in *San Francisco Bay: The Urbanized Estuary*, T. J. Conomos, Ed. (Pacific Division of the American Association for the Advancement of Science, San Francisco, 1979), pp. 47–84.
7. Two species met this latter criterion, both deliberately introduced: the signal crayfish, *Pacifastacus leniusculus*, and the muskrat, *Ondatra zibethicus*.
8. J. T. Carlton, *Ecology* **77**, 1653 (1996).
9. A. N. Cohen and J. T. Carlton, *Nonindigenous Aquatic Species in a United States Estuary: A Case Study of the Biological Invasions of the San Francisco Bay and Delta* (U.S. Fish and Wildlife Service, Washington, DC, 1995); A. N. Cohen, thesis, University of California, Berkeley (1996).
10. H. W. Graham and H. Gay, *Ecology* **26**, 375 (1945); F. P. Filice, *Wasmann J. Biol.* **16**, 159 (1959); F. A. Aldrich, *Proc. Acad. Nat. Sci. Phila.* **113**, 21 (1961); M. T. Vassallo, *Veliger* **11**, 223 (1969); Madrone Associates, *The Natural Resources of Napa Marsh* (California Department of Fish and Game, Sacramento, CA, 1977); F. H. Nichols, in *Ecology of Marine Benthos*, B. C. Coull, Ed. (Univ. of South Carolina Press, Columbia, SC, 1977), pp. 339–357; C. A. Siegfried, M. E. Kopache, A. W. Knight, *Estuaries* **3**, 296 (1980); F. H. Nichols and J. K. Thompson, *Hydrobiologia* **129**, 121 (1985); *Mar. Ecol. Prog. Ser.* **24**, 83 (1985); C. Markmann, *Tech. Rep. 12* (Interagency Ecological Studies Program for the Sacramento–San Joaquin Estuary, California Department of Water Resources, Sacramento, CA, 1986); B. Herbold and P. B. Moyle, *Biological Rep. 85(7.22)* (U.S. Fish and Wildlife Service, Washington, DC, 1989); L. Meng, P. B. Moyle, B. Herbold, *Trans. Am. Fish. Soc.* **123**, 498 (1994); Z. Hymanson, D. Mayer, J. Steinbeck, *Tech. Rep. 38* (Interagency Ecological Program for the San Francisco Bay/Delta Estuary, California Department of Water Resources, Sacramento, CA, 1994); L. Hess and D. Baty, unpublished data.
11. A. H. Arthington and D. S. Mitchell, in *Ecology of Biological Invasions*, R. H. Groves and J. J. Burdon, Eds. (Cambridge Univ. Press, Cambridge, 1986), pp. 34–53; M. N. Bruton and J. van As, in *The Ecology and Management of Biological Invasions in Southern Africa*, I. A. W. MacDonald, F. J. Kruger, A. A. Ferrar, Eds. (Oxford Univ. Press, Cape Town, 1986), pp. 47–62; P. A. Hutchings, J. T. Van der Velde, S. J. Keable, *Occas. Rep. Aust. Mus. Sydney no. 3* (1987), p. 1; D. A. Pollard and P. A. Hutchings, *Asian Fish. Sci. (Manila)* **3**, 205 (1990); *ibid.*, p. 223; A. H. Arthington, *Can. J. Fish. Aquat. Sci.* **48** (suppl. 1), 33 (1991); E. L. Mills, J. H. Leach, J. T. Carlton, C. L. Secor, *J. Great Lakes Res.* **19**, 1 (1993); K. Jansson, *Rep. 4357* (Swedish Environmental Protection Agency, Stockholm, 1994); C. F. Boudouresque, in *Introduced Species in European Coastal Waters*, C. F. Boudouresque, F. Briand, C. Nolan, Eds. (European Commission, Luxembourg, 1994), pp. 8–27; D. M. Furlani, *Tech. Rep. 5* (Division of Fisheries, Commonwealth Scientific and Industrial Research Organization, Hobart, Australia, 1996); J. A. McCann, L. N. Arkin, J. D. Williams, *Nonindigenous Aquatic and Selected Terrestrial Species of Florida* (National Biological Service, Gainesville, FL, 1996); N. C. Eno, *Aquat. Conserv. Mar. Freshw. Ecosystems* **6**, 215 (1996); M. Waldichuk, P. Lambert, B. Smiley, in *Biodiversity in British Columbia: Our Changing Environment*, L. Harding and E. McCullum, Eds. (Environment Canada, Ministry of Supply and Services, Ottawa, Ontario, 1996), pp. 220–223; E. L. Mills, D. L. Strayer, M. D. Scheuerell, J. T. Carlton, *Estuaries* **19**, 814 (1996); J. T. Carlton and M. H. Ruckelshaus, in *Strangers in Paradise: Impact and Management of Non-Indigenous Species in Florida*, D. Simberloff, D. C. Schmitz, T. C. Brown, Eds. (Island, Washington, DC, 1997), pp. 187–201; Y. Zaitsev and V. Mamaev, *Marine Biological Diversity in the Black Sea* (United Nations, New York, 1997).
12. The level of taxonomic resolution in these studies varies considerably, permitting only general comparisons. We do not count human-created aquatic systems in this comparison, such as California's Salton Sea. In addition, there has been little work on invasions in tropical shallow-water ecosystems, where high species-level diversity combined with relatively less comprehensive taxonomic study, numerous species complexes, and a reported tropicopolitan fauna may mask a high level of biological invasion.
13. L. L. Jones, *Proc. Sixth Pac. Sci. Cong.* **3**, 485 (1940); J. W. Hedgpeth, in *Between Pacific Tides*, E. F. Ricketts, J. Calvin, J. W. Hedgpeth, Eds. (Stanford Univ. Press, Stanford, CA, ed. 4, 1968), pp. 231–233; P. B. Moyle, in *Ecology of Biological Invasions of North America and Hawaii*, H. A. Mooney and J. A. Drake, Eds. (Springer-Verlag, New York, 1986), pp. 27–43. Similar arguments have been made regarding the youth and invasibility of the Baltic, Black, and eastern Mediterranean seas [see references in (*4*)].
14. E. Mayr, in *The Genetics of Colonizing Species*, H. G. Baker and G. L. Stebbins, Eds. (Academic Press, New York, 1965); L. L. Loope and D. Mueller-Dombois, in *Biological Invasions: A Global Perspective*, J. A. Drake *et al*., Eds. (Wiley, Chichester, UK, 1989), pp. 257–280; S. L. Pimm, *ibid*., pp. 351–367; T. J. Case, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 9610 (1990); M. P. Moulton, *Am. Nat.* **141**, 105 (1993); R. K. Brooke, J. L. Lockwood, M. P. Moulton, *Oecologia* **103**, 337 (1995).
15. D. Simberloff and W. Boeklin, *Am. Nat.* **138**, 300 (1991); D. M. Lodge, *Trends Ecol. Evol.* **8**, 133 (1993); D. Simberloff, in *Ecology of Biological Invasions of North America and Hawaii*, H. A. Mooney and J. A. Drake, Eds. (Springer-Verlag, New York, 1986) pp. 3–26; *Pac. Sci.* **49**, 87 (1995); P. B. Moyle and T. Light, *Ecology* **77**, 1666 (1996); R. P. Duncan, *Am. Nat.* **149**, 903 (1997).
16. It has been suggested that explosive invasions by striped bass (*Morone saxatilis*) and shad (*Alosa sapidissima*) in the 1870s and 1880s and by an Asian clam (*Potamocorbula amurensis*) in 1986–87 were facilitated by the removal of their competitors by major floods in, respectively, 1861–62 and 1985–86 [J. W. Hedgpeth, in (6), pp. 9–29; F. H. Nichols, J. K. Thompson, L. E. Schemel, *Mar. Ecol. Prog. Ser.* **66**, 95 (1990)].
17. J. T. Carlton, thesis, University of California, Davis (1979); P. B. Moyle, *Biol. Conserv.* **9**, 101 (1976).
18. R. A. Leidy and P. L. Fiedler, *Biol. Conserv.* **33**, 247 (1985). However, at least one study has found more stable habitats to be more successfully invaded [R. W. Hall and L. E. Ehler, *Bull. Entomol. Soc. Am.* **25**, 280 (1979)].
19. G. E. P. Box and G. M. Jenkins, *Time Series Analysis: Forecasting and Control* (Holden-Day, New York, 1976).

20. We thank C. Parmesan for performing the time series analysis of the rate of invasion; J. Harte, W. Berry, R. Norgaard, and two anonymous reviewers for helpful comments; and the Energy and Resources Group, University of California, Berkeley, for providing an inspiring workplace for the senior author. Supported by grants from the U.S. Fish and Wildlife Service and the National Sea Grant College Program, Connecticut Sea Grant (NOAA Grant NA36RG0467), the U.S. Fish and Wildlife Service San Francisco Bay Program, and a Switzer Foundation Environmental Leadership Grant provided through the San Francisco Foundation.

10 September 1997; accepted 9 December 1997

Role of Substrates and Products of PI 3-kinase in Regulating Activation of Rac-Related Guanosine Triphosphatases by Vav

Jaewon Han, Katherine Luby-Phelps, Balaka Das, Xiaodong Shu, Yi Xia, Raymond D. Mosteller, U. Murali Krishna, John R. Falck, Michael A. White, Daniel Broek*

Mitogen stimulation of cytoskeletal changes and c-jun amino-terminal kinases is mediated by Rac small guanine nucleotide-binding proteins. Vav, a guanosine diphosphate (GDP)–guanosine triphosphate (GTP) exchange factor for Rac that stimulates the exchange of bound GDP for GTP, bound to and was directly controlled by substrates and products of phosphoinositide (PI) 3-kinase. The PI 3-kinase substrate phosphatidylinositol-4,5-bisphosphate inhibited activation of Vav by the tyrosine kinase Lck, whereas the product phosphatidylinositol-3,4,5-trisphosphate enhanced phosphorylation and activation of Vav by Lck. Control of Vav in response to mitogens by the products of PI 3-kinase suggests a mechanism for Ras-dependent activation of Rac.

Mitogens induce Rac-mediated changes in the actin cytoskeleton as well as Rac-mediated regulation of gene expression. Rac-related proteins are activated by a family of guanine nucleotide exchange factors (GEFs) related to the Dbl oncogene product that catalyze the exchange of bound GDP for GTP on Rac-related guanosine triphosphatases (GTPases) (*1*, *2*). The functional properties of the Dbl homology domain of the Vav oncogene product are regulated by Lck-dependent tyrosine phosphorylation (*3*, *4*). However, this observation alone does not explain the apparent interaction of Vav with the Ras signaling pathway (*5*, *6*). T cell receptor activation results in activation of Vav, Ras, and PI 3-kinase (*7*). Each of these events results in activation of Rac-related GTPases (*3*, *4*, *8*). However, the mechanism by which Ras, PI 3-kinase, and Vav might interact to mediate activation of Rac is unknown. Vav, like all known Dbl-related molecules, has a pleckstrin homology (PH) domain on the COOH-terminal side of the GEF domain (*1*). Because some PH domains bind substrates and products of PI 3-kinase (*9*–*11*), we tested whether phosphoinositides bound to the PH domain of Vav and affected Vav GEF activity for Rac-related GTPases.

We examined the ability of Vav to stimulate guanine nucleotide exchange on Rac in the presence of substrates or products of PI 3-kinase. We used water-soluble analogs of phosphatidylinositides that are biologically active (*12*, *13*). A His-tagged Vav fusion protein was incubated with the Lck tyrosine kinase to allow the kinase reaction to go to completion. Lck was removed by successive washings of the immobilized Vav protein. Phosphorylated Vav, but not unphosphorylated Vav, stimulated the release of [^{3}H]GDP from Rac (Fig. 1A) (*3*). The presence of a water-soluble PI 3-kinase substrate, C8 phosphatidylinositol-4,5-bisphosphate [C8PtdIns(4,5)P_2], in the GEF reaction resulted in a 90% inhibition of Vav GEF activity. The natural PtdIns(4,5)P_2, when incorporated into micelles, also inhibited Vav-GEF activity to a similar extent (*14*). In contrast, the presence of PI 3-kinase products, C8 phosphatidylinositol-3,4-bisphosphate [C8PtdIns(3,4)P_2] or C8 phosphatidylinositol-3,4,5-trisphosphate [C8 PtdIns(3,4,5)P_3], in the GEF reaction resulted in a twofold stimulation of Vav GEF

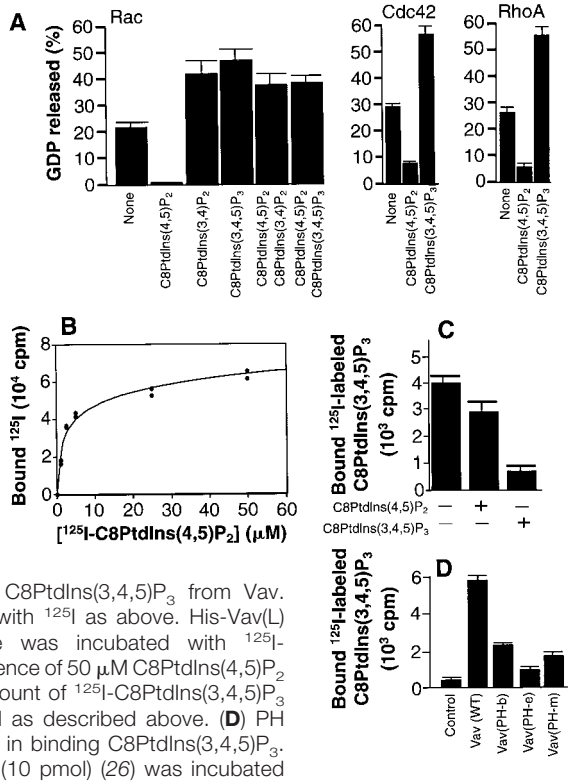

Fig. 1. Interaction of phosphoinositides with the PH domain of Vav and modulation of Vav GEF activity. (**A**) Regulation of Vav GEF activity in vitro. Lck-phosphorylated Vav was incubated with Rac, Cdc42, or RhoA and C8PtdIns(4,5)P_2 (10 μM), C8PtdIns(3,4)P_2 (10 μM), or C8PtdIns(3,4,5)P_3 (10 μM) (*12*). Guanine nucleotide release assays were done as described (*3*). (**B**) Dose-dependent binding of ^{125}I-labeled C8PtdIns(4,5)P_2 to Vav. His-tagged Vav(L) protein (*3*) immobilized on Ni-agarose was incubated with the indicated concentration of Bolton-Hunter reagent–labeled C8PtdIns(4,5)P_2-NH_2 (*25*). Material associated with His-Vav(L) immobilized on Ni-agarose was collected, and the amount of ^{125}I-phosphoinositide was counted with a gamma counter. (**C**) Failure of C8PtdIns(4,5)P_2 to displace C8PtdIns(3,4,5)P_3 from Vav. C8PtdIns(3,4,5)P_3-NH_2 was labeled with ^{125}I as above. His-Vav(L) protein immobilized on Ni-agarose was incubated with ^{125}I-C8PtdIns(3,4,5)P_3 alone or in the presence of 50 μM C8PtdIns(4,5)P_2 or 50 μM C8PtdIns(3,4,5)P_3. The amount of ^{125}I-C8PtdIns(3,4,5)P_3 associated with Vav was determined as described above. (**D**) PH domain mutants of Vav are defective in binding C8PtdIns(3,4,5)P_3. Wild-type or mutant His-Vav protein (10 pmol) (*26*) was incubated with a saturating amount of ^{125}I-C8PtdIns(3,4,5)P_3, and the amount of C8PtdIns(3,4,5)P_3 binding to Vav was determined as above.

J. Han, B. Das, X. Shu, Y. Xia, R. D. Mosteller, D. Broek, Department of Biochemistry and Molecular Biology, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA 90033–0800, USA. K. Luby-Phelps and M. A. White, Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235–9039, USA.
U. M. Krishna and J. R. Falck, Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235–9039, USA.

*To whom correspondence should be addressed. E-mail: broek@zygote.hsc.usc.edu