Clotting Protein Cloned

Three groups of researchers have cloned the gene for von Willebrand factor and now plan to investigate how the protein functions and how it is made

Most hematologists agree that of all the factors that go into the complex chemical cascade that results in blood clotting, two are of special interest. The absence of either of these factors results in the two most common bleeding disorders—hemophilia and von Willebrand's disease. Now the genes for both of these protein factors have been cloned.

Last fall, researchers at Genentech in San Francisco and, independently, researchers at the Genetics Institute in Boston reported in the 22 November issue of *Nature* that they had cloned factor VIIIC, the protein that is absent in hemophiliacs. And now three groups have independently cloned the gene for von Willebrand factor. One of these groups, from Children's Hospital and Brigham and Women's Hospital at Harvard Medical School, reports on its work in this issue of *Science* (p. 1401). Another, from the Dana Farber Cancer Institute, also at Harvard Medical School, describes its work in the May issue of *Cell*. The third, from Washington University and the University of Washington, has a paper in press at *The Proceedings of the National Academy of Sciences*.

Von Willebrand factor, like factor VIIIC, is a huge protein that is of clinical significance as well as theoretical importance. The protein is of interest to hematologists because it is involved in two different aspects of blood clotting—platelet adherence and the formation of the actual blood clot. And because von Willebrand factor is necessary for platelets to adhere to blood vessel walls and because platelet adherence is thought to play a role in the development of atherosclerosis, some investigators speculate that people with von Willebrand's disease may be protected against heart disease.

People with von Willebrand's disease bleed for a long time if they are cut and women with the disease have heavy menstrual bleeding. Most von Willebrand disease patients have one normal gene for the factor and one abnormal gene. They make about half the usual amount of the factor and their disease is mild. Nonetheless, hematologists suspect it is the most common bleeding disorder, with many patients going undiagnosed.

Severe von Willebrand's disease is much rarer—Harvey Weiss of Columbia University College of Physicians and Surgeons estimates its incidence at 1 in a million. But these patients are severely affected. They have virtually no von Willebrand factor; they bleed uncontrollably when they are injured and are particularly prone to serious episodes of gastrointestinal bleeding. In addition, they have some symptoms of hemophilia. Von Willebrand factor somehow regulates the plasma levels of factor VIIIC; people with severe von Willebrand's disease lack both von Willebrand factor and are deficient in factor VIIIC.

> The protein is involved in two different aspects of blood clotting.

Hemophiliacs bleed into their joints, causing pain and disfigurement; sometimes they have serious internal bleeding as well. The clotting factor they are missing, factor VIIIC, is necessary to form the fibrin clot that stops bleeding until tissue can be repaired. But hemophiliacs do not bleed uncontrollably when they are cut, perhaps, hematologists propose, because "tissue factors" can substitute for factor VIIIC. If they bleed into a joint, however, there is, presumably, very little of these tissue factors around and so the bleeding may continue until factor VIIIC is medically supplied.

Blood clotting has essentially three components. When a person is cut, the first thing that happens is that platelets adhere at the site, release their contents, and aggregate to form a plug. This process requires von Willebrand factor and is the reason why people with even mild von Willebrand's disease bleed for abnormally long periods. Next, a complex sequence of reactions occurs on the surface of the platelet plug and on the surrounding blood vessel wall, resulting in the formation of a fibrin clot, which seals the cut while the tissue is repaired. Finally, when the tissue is repaired, the clot is broken down.

One reason the cloned clotting factor genes are so interesting, says Stuart Orkin of the Children's Hospital group that cloned von Willebrand factor gene, is that they will enable investigators to finally understand just what the relationship is between factor VIIIC and von Willebrand factor. For years, there was some controversy over whether the two factors were even distinct, leading to what Orkin terms "the mystique over the 'factor VIII complex.' " But now investigators realize that the confusion was caused by the way the factors are associated. They travel together in the blood in a complex that is 99 percent von Willebrand factor. Originally, when researchers used antibodies to measure factor VIIIC levels, they mistakenly measured von Willebrand factor instead because their antibodies were directed against the larger protein complex, according to David Ginsburg of the Brigham team.

Weiss and others would like to know why von Willebrand factor seems to regulate plasma levels of factor VIIIC. One possibility—and one that is Weiss' own prejudice—is that von Willebrand factor stabilizes factor VIIIC. This occurs in vitro and it should now be possible to learn if it happens in vivo as well. The experiment would be to inject highly purified factor VIIIC into hemophiliacs and into persons with severe von Willebrand's disease. If von Willebrand factor stabilizes factor VIIIC, the injected factor would last longer in the hemophiliacs who make normal quantities of von Willebrand factor.

In addition, Orkin points out, it should now be possible to study the interaction between factor VIIIC and von Willebrand factor on a molecular level and to investigate which parts of the molecules are important to their interaction. This might also be clinically significant because if factor VIIIC is stabilized by its interaction with von Willebrand factor, it may be necessary to provide both cloned molecules to hemophiliacs.

Ginsburg and his colleagues hope to use von Willebrand factor complementary DNA to investigate the inheritance of von Willebrand's disease and, as a side issue, to look for a possible link between von Willebrand's disease and protection from atherosclerosis. "Von Willebrand factor is involved when platelets stick to injured blood vessels.

Slime Molds on the Wing

It is no small irony that although the biochemistry, genetics, and developmental biology of the cellular slime molds are relatively well explored, knowledge of the natural history of these popular experimental organisms remains patchy at best. However, owing to the fortuitous combination of two diverse interests—those of slime mold biology and the habits of migratory songbirds—in a Princeton researcher, Hannah Bonsey Suthers, one curious aspect of this natural history appears to have been solved: to wit, the very broad geographical distribution of cellular slime molds. Suthers reports that ground-feeding, migratory songbirds play a major role in carrying these microscopic organisms great latitudinal distances in the Americas and presumably elsewhere (*1*). Although birds are known to be important in the dispersal of higher plants, mosses, and fungi, this is the first demonstration that they also influence the natural history of slime molds.

The life cycle of slime molds involves a stage at which free-living, dispersed amoebas forage independently, followed by an aggregation stage in which the amoebas come together to form a mobile slug, which behaves much like a tiny multicellular organism. After migrating, the slug forms a fruiting body, which can contain several hundred spores. Now, although the slug is mobile, its range is limited: 1 inch in 24 hours is typical. As far as is known, more widespread dispersal of spores occurs by attachment to passing insects (by electrostatic attraction) and by water; both routes are relatively restricted in geographical terms. The discovery that birds may passively carry propagules of slime molds immediately extends the range of potential dispersal. What is of interest here, however, is the distribution of single species over thousands, not merely hundreds, of miles.

Cellular slime molds are remarkable both in the very small number of species in the group—50 as compared with 500 in the myxomycetes, for example—some of which have worldwide distribution. These organisms are very ancient in evolutionary terms, and a global distribution could therefore simply be a consequence of having been passive passengers on landmasses that periodically coalesce and fragment. But the fact that single species are recognizable across several continents implies a continual interchange between populations, because geographically isolated populations would be expected to diverge over relatively modest tracts of geological time.

The initial steps of Suthers's study involved culturing the droppings of ground-feeding birds, simply to see if slime molds could be found there. After showing that they could, she began a systematic survey of slime mold species that could be found in ground-feeding, migratory birds in New Jersey and Central America, which variously represent summer or winter homes for many songbirds. Comparison of slime mold species from droppings with those cultured from the soil surface throughout the seasons shows clear pulses of immigration of slime molds as a result of the various northerly and southerly avian migrations.

Unlike the seeds of higher plants, which pass through birds relatively quickly, slime mold propagules, particularly the resilient spores, can persist as long as 10 days. As the journey from the northeast coast of North America to the Caribbean and South America is accomplished in just 72 hours by millions of songbirds every fall, the potential for long-distance dispersal is obvious. The return journey in the spring is a more leisurely affair of some 20 days, which effects a different pattern of dispersal.

Although this study explains the great latitudinal dispersals in the Americas, it leaves unsolved the distribution of a recently discovered cellular slime mold species, *Polysphondylium filamentosum* (*2*), which occurs in the Swiss Alps, Central America, and, albeit rarely, in North America, apparently transported there by the ovenbird. Suthers notes that no ground-feeding bird routinely makes a trans-Atlantic migration that could be responsible for this distribution.—**ROGER LEWIN**

References

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2. F. Traub, H.R. Hohl, J.C. Cavender, *Amer. J. Bot.* **68**, 162 (1981).

Many people think that is the first step in the development of atherosclerotic plaques," Ginsburg explains. A strain of pigs with von Willebrand's disease is protected against heart attacks, even if the pigs are fed a high fat diet which causes normal pigs to get atherosclerosis and heart attacks.

Ginsburg speculates that certain variants of von Willebrand factor could conceivably be an atherosclerosis risk factor. People who have a slightly overactive protein, for example, might be at increased risk. A few years ago, the National Heart, Lung and Blood Institute decided to investigate the proposed link between von Willebrand factor and heart disease by compiling a registry of von Willebrand's disease families and following them to see if they had an unusually low incidence of heart disease, but the project proved too expensive and difficult to do and so it has not been completed. A European and Israeli group, the European Thrombosis Research Organization, also is looking at the incidence of heart disease in people with severe von Willebrand's disease. But Ginsburg and his associates hope to get the answer by reversing the strategy.

Using a molecular probe for von Willebrand factor, they plan to look at families with a high incidence of atherosclerosis to see if heart disease is linked with the inheritance of particular von Willebrand factors. "These studies are doable," Ginsburg remarks. "We can take a DNA probe and look for polymorphisms that are linked to von Willebrand factor. That gives us a way to look at DNA in families and see if a von Willebrand gene is a risk factor. We have already found polymorphisms for von Willebrand factor and now we are starting to look at families." Nonetheless, Orkin cautions, this research is very preliminary and, he remarks, the chances of a payoff are still slim. "My own view is that it's a long shot," he says.

All three groups that cloned the von Willebrand factor gene say they are primarily interested in using the gene to study how the protein is synthesized and how its synthesis is regulated. Dennis Lynch of the group at Dana Farber that cloned the von Willebrand factor gene and, independently, Evan Sandler of the Washington University group point to a number of unanswered questions. For example, the factor is made only in megakaryocytes, which are bone marrow cells that are precursors of platelets, and in endothelial cells. "If we could get the appropriate regions of the gene, we could look at why the gene is only expressed in those two cell types," says Sandler.

Lynch wants to know if the messenger RNA for the von Willebrand factor made in endothelial cells is even the same as the message made in megakaryocytes. "In megakaryocytes, von Willebrand factor is stored as multimers in the cells. In endothelial cells, the bulk of the protein is in the form of dimers. Why is that? Is it exactly the same protein or is it a slightly different form?" he asks.

A final consequence of having the cloned von Willebrand gene is that investigators should be able to sort out at last the confusing variations of von Willebrand's disease. "Von Willebrand's disease is the thalassemia of coagulation," says Ginsburg. In thalassemia, there were multiple subtypes and variants, all clinically defined and hard to understand until molecular biologists, including Orkin, analyzed the genes involved and were able to explain the thalassemia variants on a molecular level.

"Hemophilia is fairly straightforward," says Ginsburg. "In general, the less factor VIIIC activity you have, the more severe the disease. But with von Willebrand's disease, there are all different kinds of subtypes. Type I is most common, but now there are types Ia, Ib, and Ic. There also are types IIa, IIb, and IIc as well as a type III." People with type II von Willebrand's disease, for example, make normal amounts of the protein but the protein itself is abnormal. Some type II's make proteins that do not aggregate properly and others make proteins that are overly active and bind too tightly to platelets. "It's a real morass," Ginsburg remarks.

But with the cloned gene in hand, molecular biologists should be able to pinpoint the causes of the different subtypes of von Willebrand's disease, making the classification of the subtypes more rational and diagnosis easier. In addition, the subtypes undoubtedly include mutant proteins that are not processed properly, and by studying them, investigators should learn how the von Willebrand factor is processed. Already, they know it starts out as a 300,000 dalton precursor and ends up as 220,000 dalton subunits which then aggregate to form complexes with molecular weights as high as 20 million. By studying the various mutant proteins, they should be able to learn what happens to this protein along the way to its final destination in the blood.—**GINA KOLATA**

Los Alamos Neutron Source Meets First Test

Storing a beam in the proton storage ring means a world-class source is in sight, but money for an experimental hall is lacking

26 April was a day for celebrating at the Los Alamos National Laboratory as jubilant researchers stored a beam in the Proton Storage Ring (PSR) on the very first attempt. The PSR is a $22-million addition to the Weapons Neutron Research (WNR) facility whose effect will be to convert the WNR into a world-class pulsed source for neutron scattering on a par with the British Spallation Neutron Source that recently entered the commissioning phase at the Rutherford Appleton Laboratory (*1*). The combined WNR/PSR facility will be dedicated this August as the Los Alamos Neutron Scattering Center (LANSCE).

However, two important provisos to be satisfied before researchers can tap the intense neutron beam the WNR/PSR will provide are the construction of an experimental hall sufficiently large to house research instrumentation and the development of the instrumentation itself. Now set at $17.5 million, funding for this purpose has never made it into the presidential budget. "Without a substantial increase in money, Los Alamos will have a first-rate source of neutrons that can't be effectively used," sums up J. Michael Rowe of the National Bureau of Standards (NBS), which itself has been trying to obtain support for a research facility for very long wavelength (cold) neutrons around its own reactor-based neutron source.

This year the House Committee on Science and Technology, in its markup of the Department of Energy (DOE) civilian research and development authorization bill, directed DOE to begin funding construction of the experimental hall in fiscal 1986. The committee authorized $1 million for this purpose (to be accommodated by a decrease elsewhere in DOE's budget), with a total of $18.4 million to be reached during the following 2 years. Whether these directives survive in the full House or in the Senate and whether funds are ultimately provided in the all-important companion appropriations bills are, of course, uncertain given the widespread concern over the national budget deficit.

The WNR/PSR is important to U.S. neutron scatterers because it addresses the two most important issues facing the field: providing facilities equal to those of researchers elsewhere in the world and laying the groundwork for the higher intensity neutron source that will be needed in the 1990's to replace the reactors that are the mainstays of the present program.

Driving much of the concern is the specter of European and, increasingly, Japanese competition. Observers generally agree that, starting in the 1970's, European researchers have gradually wrested away leadership in neutron scattering from their American colleagues. The British-French-German Institut Laue-Langevin (ILL) in Grenoble, whose reactor started up in 1971 and whose budget alone matches that of the entire U.S. neutron scattering program, symbolizes that leadership.

With a flux of 1.5×10^{15} thermal neutrons per square centimeter per second (neutrons/cm^2-sec), the ILL reactor is no more intense than comparable reactors at Brookhaven National Laboratory and Oak Ridge National Laboratory. But a combination of a more versatile reactor design, large numbers of experienced scientists, and adequate resources has allowed the maximum exploitation of the available neutrons. Special moderators (cold sources) generate very low energy neutrons that give details of large complex molecules (polymers and biological macromolecules) not obtainable with ordinary thermal neutrons. Guide tubes coated with neutron-reflecting material transport the neutrons large distances, allowing more instruments around the reactor and in some cases greatly enhancing resolution. And instruments of improved resolution and sensitivity effectively multiply the neutron flux.

Moreover, the ILL is hardly the whole show. According to a recent compilation by Roger Pynn and Brian Fender of the ILL, while that facility has 26 neutron scattering instruments surrounding its reactor at present (seven more will be