ure). R_{eleven} is at least an order of magnitude bigger than $R_{Calabi\text{-}Yau}$ and may indeed be much larger. If we disregard the tiny six-dimensional Calabi-Yau manifold, the remaining five-dimensional space-time contains two flat mirror planes and closely resembles the ideas of Kaluza and Klein. In fact each mirror plane is a static 3-brane, one of which is our world and the other the world of what phenomenologists call the "hidden sector," the world of matter which interacts only weakly with our own, essentially via gravitational forces.

This static compactification model of Horava and Witten has come to replace the older model of Candelas, Horowitz, Strominger, and Witten (*1*) but it still has its limitations. Nevertheless, the basic idea that at least some of the extra dimensions could be quite large has caught on, together with the suggestion (*7*, *8*) that the basic length scale of quantum gravity, which hitherto has been equated to Planck's constant or to the slightly smaller length scale R_{String} of superstring theory, may be much larger, more like the electroweak scale of 10^{-17} cm. In principle this could allow extra dimensions approaching a millimeter! The string scale and the size of the extra dimensions and Newton's constant are related by a simple ratio. An experimental limitation on the scale of the extra dimension now arises from the accuracy with which gravity obeys the inverse square law.

Even more radically, Randall and Sundrum have recently revived the old dream that one may be able to dispense with the compactification hypothesis altogether and contemplate our universe as an isolated 3-brane moving in an infinitely large space-time (*9*). Randall and Sundrum's five-dimensional universe still has a small characteristic radius curvature, but their universe is truly infinite in all four spatial dimensions. The matter of which we are made up is confined to the 3-brane, rather like beads on a wire. Gravity acts in all dimensions but one of the achievements of Randall and Sundrum was to show that gravity can act in the correct way in our three-dimensional world. Before their work, no one believed that such a model was consistent with Newton's conventional inverse square law for the gravitational force, but Randall and Sundrum appear to have convinced most of their critics on this score.

Much needs to be done to produce a completely satisfactory cosmology. But Randall and Sundrum and the many others currently working in this field have clearly demonstrated that brane-world scenarios using small extra dimensions make testable predictions, both for nucleosynthesis during the Big Bang and for the existence of new particles, for cosmologists and phenomenologists to refute or confirm.

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PERSPECTIVES: EPIDEMIOLOGY

Tracing the Origins of *Salmonella* Outbreaks

Andreas J. Bäumler, Billy M. Hargis, Renee M. Tsolis

In the 1980s, health officials in Europe and the Americas noted a considerable increase in human food-borne illness caused by *Salmonella enteritidis*, a pathogen found in chicken carcasses, eggs, and egg products (*1*). It has been suggested that the emergence of *S. enteritidis* as a public health problem may be the result of modern poultry farming practices and of a decline in the genetic diversity of domestic fowl (*2*). But this hypothesis does not explain why the number of humans infected with other *Salmonella* serotypes, such as *S. typhimurium*, has not increased (*3*, *4*). For example, the incidence of human infection with *S. enteritidis* has steadily increased since the 1960s, whereas the incidence of *S. typhimurium* infection has remained relatively constant (see figure, this page). It has been proposed that the *S. enteritidis* epidemic could be caused by clonal expansion of a single, more virulent *S. enteritidis* isolate. However, the observation that human *Salmonella* cases in Europe and the United States are associated with different *S. enteritidis* isolates does not support this notion.

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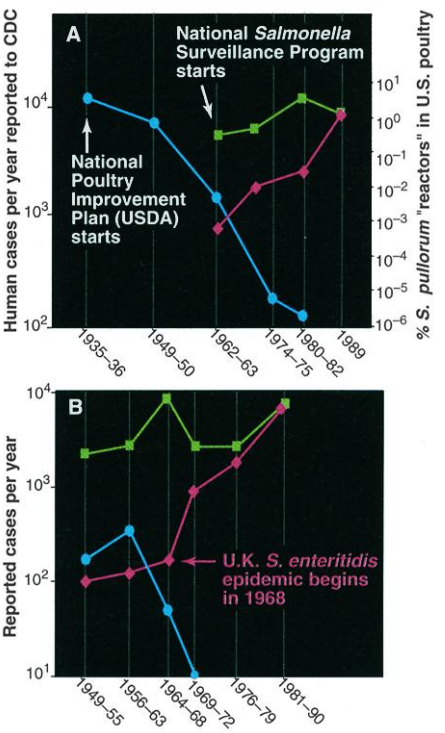
Through a retrospective analysis of epidemiological surveys, we now put forward the hypothesis that the epidemic of salmonellosis in humans due to *S. enteritidis* was triggered by this *Salmonella* serotype filling the ecological niche vacated by the avian *Salmonella* pathogens *S. pullorum* and *S. gallinarum*. Retrospective analysis revealed that *S. enteritidis* became established in poultry flocks in the 1960s, which coincided with the eradication of the avian *Salmonella* pathogens from do-



Secrets of *Salmonella* serotypes. Prevalence of *Salmonella* serotypes in humans and poultry in the United States (**A**) and in England and Wales (**B**). Circles show the percentage (A) or incidence (B) of domestic poultry infected with *S. pullorum* (*17*, *18*). Squares and diamonds show the numbers of human cases of food-borne illness caused by *S. typhimurium* or *S. enteritidis*, respectively (*3*, *8*, *9*, *19*).

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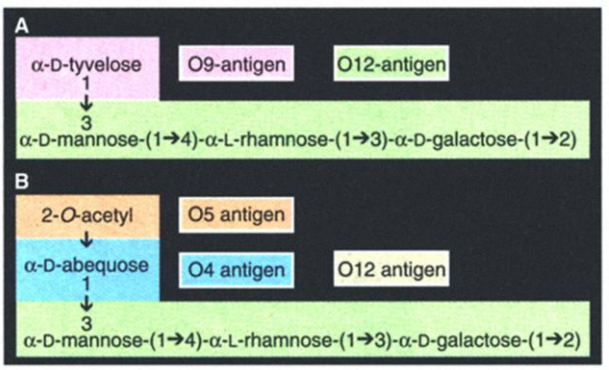
mestic fowl. As these three pathogens share a common immunodominant surface antigen (O9), we postulate that the flock immunity generated by the two avian *Salmonella* biotypes prevented *S. enteritidis* from circulating in poultry flocks in the first half of this century.

Records documenting the prevalence of *Salmonella* serotypes among poultry in Britain and North America date back to 1930. At this time, an illness called "bacillary white diarrhea" (pullorum disease), caused by *S. pullorum*, posed a serious economic threat to the poultry industry (*5*). A test tube agglutination assay revealed that 10 to 20% of chicks possessed high titers of antibody against *S. pullorum* (*6*). A National Poultry Improvement Plan, designed to reduce the level of *S. pullorum* infection in the United States, was adopted in 1935 and included large-scale voluntary testing of poultry flocks using a whole-blood test for agglutination of stained antigen. Similar *S. pullorum* control programs were implemented in some European countries. In 1954, the National Poultry Improvement Plan was revised to recognize fowl typhoid, an illness caused by *S. gallinarum* (*5*). As both biotypes belong to the same *Salmonella* (O9) serotype, blood testing for pullorum disease effectively lowered the incidence of fowl typhoid as seropositive birds were culled. Thus, by the mid-1970s, *S. pullorum* and *S. gallinarum* were eliminated from commercial poultry flocks in Britain and the United States (see figure, previous page).

In the early 1900s, human *S. enteritidis* infections were not associated with poultry, but rather with rodents, the only known animal reservoir for this pathogen (*7*). This situation changed in the second half of this century. The incidence of *S. enteritidis* infection in the British population remained almost constant between 1949 and 1963, but the increased frequency of *S. enteritidis* infection of chickens (which began in 1967) resulted in a doubling of human cases reported in 1968 and 1969 and a continued increase thereafter (*8*, *9*) (see figure, previous page). Similarly, the first 5-year report on human salmonellosis in the United States revealed that *S. enteritidis* increased in frequency from being the sixth most common causative serotype in 1963 to the third most common in 1967 (*3*). When these data are plotted on a linear scale, the beginning of the epidemic is not apparent because the exponential increase in *S. enteritidis* cases produces a sigmoid curve. However, logarithmic conversion of the data shows that the number of human cases has increased at an almost constant rate since the 1960s (see figure, previous page). Thus, it seems possible that the current *S. enteritidis* epidemic started in the late 1960s—much earlier than previously thought (*1*).

It is likely that *S. enteritidis* was initially introduced into poultry flocks through its rodent animal reservoir because mice and rats captured in hen-houses frequently carried this organism (*10*). But, as *S. enteritidis* was present in rodents when monitoring began in the 1930s (*7*), it is not obvious why it did not spread to domestic fowl until much later. An important event coinciding with the increase in human *S. enteritidis* cases in Britain and the United States in the 1960s, was the remarkable decline in domestic poultry that tested seropositive for *S. pullorum*. We suggest that the elimination of *S. pullorum* "reactors" may have increased the ability of *S. enteritidis* to gain a foothold in poultry flocks. The O antigen of *S. enteritidis*, *S. pullorum*, and *S. gallinarum* consists of the O12 antigen (a sugar backbone composed of O-polysaccharide repeating units) and the O9 antigen (a tyvelose sugar chain) (see figure, this page). Chickens infected with *S. gallinarum* or *S. pullorum* develop O9 antibody titers that are >10-fold higher than O12 antibody titers (*11*), suggesting that the O9 antigen is immunodominant. Consistent with the induction of protective immunity by O9 antigen, vaccination with live *S. gallinarum* (O9,12) protects mice against subsequent challenge with *S. enteritidis* (O9,12) but not against challenge with virulent *S. typhimurium* (O4,5,12) (*12*). Furthermore, vaccination of mice with an *S. enteritidis aroA* mutant (O9,12) elicits protection against subsequent challenge with a virulent *S. typhimurium* strain genetically engineered to express the O9,12 antigen but not against wild-type *S. typhimurium* (O4,5,12) (*13*). Thus, cross-immunity is, at least in part, caused by an immune response directed against the immunodominant O9 antigen. We propose that seropositive *S. pullorum* poultry had increased immunity that protected them against infection with *S. enteritidis*; this could have reduced the transmission of this biotype by reducing the number of susceptible hosts (*14*). So, flock immunity against the O9 antigen generated by the avian *Salmonella* biotypes could have prevented *S. enteritidis* from circulating in the avian host population. After elimination of seropositive birds from European and U.S. commercial poultry flocks in the 1960s, *S. enteritidis* would then have readily been able to circulate among domestic fowl. The increased incidence of salmonellosis in humans may have been caused by *S. enteritidis* filling the ecological niche vacated by eradication of the avian pathogens. The number of *S. typhimurium* cases, on the other hand, would not be expected to increase because this serotype lacks the O9 antigen and therefore would be unaffected by this elimination.



Sugary coat of an evil pathogen. Chemical composition of the O antigen of different *Salmonella* serotypes. (**A**) The O antigen of *S. enteritidis*, *S. gallinarum*, and *S. pullorum* consists of an O12 backbone of sugar repeats and O9 (a tyvelose branching sugar). (**B**) The O antigen of *S. typhimurium* has the same O12 backbone but different (O4,5) branching sugar chains.

Although *S. enteritidis* possesses the O9 antigen, its establishment in poultry flocks was not detected or prevented by *S. pullorum* surveillance programs. Because *S. enteritidis* colonizes chickens without causing overt signs of disease (and hence without eliciting high anti-O9 titers), it escapes detection by the relatively insensitive whole-blood agglutination test (*15*). The lack of simple and inexpensive methods for detecting *S. enteritidis* in poultry has been a major impediment to implementing effective control measures (*16*). However, even with a good test in place, it is unlikely that the current epidemic could be controlled, because unlike avian *Salmonella* pathogens, *S. enteritidis* has a rodent animal reservoir. Therefore, culling infected birds, which proved so successful during the eradication of *S. gallinarum* and *S. pullorum*, has a much smaller chance of success in reducing the incidence of *S. enteritidis* in poultry. Instead, a more effective strategy might be to reestablish flock immunity through vaccination.

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PERSPECTIVES: BIOCHEMISTRY

Reading the Worm Genome

Stuart K. Kim

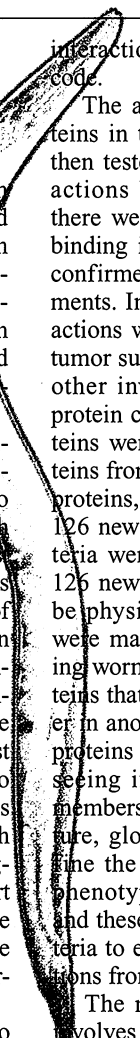
A powerful, top-down, holistic approach in biological research is to identify all of the components of a particular cellular process, so that one can define the global picture first and then use it as a framework to understand how the individual components of the process fit together. On page 116 of this issue, Walhout *et al.* report that they have started to compile a global map of interactions between all of the proteins in the worm *Caenorhabditis elegans* (*1*). These investigators commandeered a small number of well-studied proteins to establish the technical and conceptual framework for this mammoth protein-binding project. Their ultimate goal is to illuminate all of the protein-protein interactions in this animal, and to combine this information with that from other functional genomics approaches to work out what each worm gene does.

There is a rich scientific history of applying global approaches to biological problems. In the early 1960s, all of the genes in the bacteriophage were identified with saturation genetic screens (*2*). In 1983, the complete cell lineage of *C. elegans*—the 969 cells that constitute its developmental program and the 302 neurons that control its behavior—was determined (*3*). Monumental studies have elucidated the complete genome sequence of the yeast and nematode, and identified all of their genes (*4*). Recently, DNA microarrays have profiled the expression pattern of every gene in yeast, providing a complete molecular fingerprint of the cell cycle and sporulation program in this organism (*5*).

Walhout and colleagues chose the nematode as a model to study protein-protein interactions. Of the worm's 19,293 predicted proteins, only 7% (encoded by 1277 genes) have been studied at either the genetic or biochemical level by the *C. elegans* research community. Clearly, to characterize the remaining 93% of worm proteins, Walhout and co-workers needed to develop a global approach rather than analyzing the interactions of each individual protein. They developed a high-throughput method to analyze proteins in parallel that can be automated and should be suitable for the study of protein-binding interactions across the entire genome.

Their approach to identifying protein-binding interactions is based on the well-established yeast two-hybrid system. Two worm proteins are expressed in yeast, each one connected to different halves of a yeast transcription factor. If the worm proteins bind to each other, they bring the halves of the transcription factor together, resulting in expression of a selectable marker. The investigators used bacterial recombinase enzymes to accurately insert their polymerase chain reaction (PCR) fragments into yeast two-hybrid vectors. This cloning step was so efficient that most of the resulting clones contained the correct cDNA fragment. Both the PCR step to produce the cDNA fragments and the recombination step to insert the fragments into the yeast vectors can be automated. Thus, the entire project can be scaled up to eventually address protein interactions across the whole genome.

Conceptually, Walhout *et al.* needed to show that the yeast two-hybrid method could confirm protein interactions that were already known. In addition, they laid down guidelines to evaluate whether newly identified protein interactions were physiologically significant. They chose to use genes involved in vulval development as a test case to evaluate the suitability of the yeast two-hybrid approach. Vulval development has been extensively studied and is known to be regulated by at least four major sets of genes. These genes encode components of the RTK/Ras/MAP kinase, Notch and Wnt signaling pathways and the retinoblastoma (Rb) tumor suppressor protein complex. More than 50 genes involved in vulval development have been characterized, and there is a large amount of genetic and biochemical data available to confirm interactions among the proteins they encode.



The authors expressed 27 vulval proteins in the yeast two-hybrid system and then tested whether there were any interactions between them. Among the 27, there were 11 previously known protein-binding interactions, and 6 of these were confirmed in the yeast two-hybrid experiments. In addition, two new protein interactions were found, one involving the Rb tumor suppressor protein complex and another involving a receptor localization protein complex. Next, the 27 vulval proteins were used to select interacting proteins from a large library of random worm proteins, resulting in the identification of 126 new protein interactions. Several criteria were used to indicate which of the 126 new interactions were more likely to be physiologically relevant. First, there were many examples where two interacting worm proteins were homologs of proteins that were known to bind to each other in another species. Second, some worm proteins were independently identified by seeing if they interacted with different members of a protein complex. In the future, global approaches are likely to define the expression patterns and mutant phenotypes of large numbers of genes, and these data will serve as additional criteria to evaluate predicted protein interactions from these experiments.

The most striking set of interactions involves the Rb tumor suppressor protein complex, which regulates gene expression during the cell cycle. Protein expression libraries in the yeast two-hybrid system were screened with four worm proteins (LIN-15, LIN-36, LIN-37, and the RbAp48 homolog) known to have repressor functions similar to Rb (they are encoded by genes that when mutated induce similar phenotypes to that of the mutant Rb gene). These screens yielded 10 interacting proteins, three of which were homologs of mammalian proteins known to be part of the Rb protein complex (Rb itself, HDAC, and MTA1). Each of the remaining seven specifically interacted with two different proteins in the Rb complex, strongly suggesting that these new proteins are components of the Rb complex.

The *C. elegans* protein interaction map

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