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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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can be viewed at two levels. First, it will help to elucidate gene function on a global scale. For those proteins that are conserved during evolution, interactions between two worm proteins may clear the way to finding homologous interactions in mammals. For example, some of the new worm proteins that interact with the Rb complex are conserved, and their homologs might also be part of the Rb complex in mammals. Second, along with all of the other functional genomics projects in *C. elegans*, the protein interaction map will serve as a "model of models" to establish the experimental logic and techniques that will need to be developed to interpret the overwhelming amount of data generated by genome sequencing. If genomes are like books, then all of the words (19,293 genes) in the worm book are already known, and their definitions should soon be clarified by functional genomics. The grammar used to construct genetic pathways is simpler in worms than in mammals because there are fewer words, fewer synonyms and simpler sentence structures in the worm genome (that is, fewer genes, less genetic redundancy and fewer feedback loops). Thus, to learn how to read the book of an organism's genome, it makes sense to start with the worm.

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PERSPECTIVES: PLANETARY SCIENCE

Charon's First Detailed Spectra Hold Many Surprises

Eliot Young

Pluto and its satellite Charon are the two brightest members of the Kuiper Belt, that no-longer exclusive club of objects orbiting the sun beyond the orbit of Neptune (*1*). The Pluto-Charon system is expected to be a fairly pristine reservoir of the outer solar system's volatile materials, although some processing does occur: ultraviolet (UV) photolysis, charged particle bombardment, impacts by meteorites or comets, and (in the case of Pluto) wholesale resurfacing through periodic condensation and sublimation of frosts. But Pluto and Charon continue to surprise us at every turn, and the most recent spectra of Charon on page 107 of this issue by Brown and Calvin (*2*) are no exception.

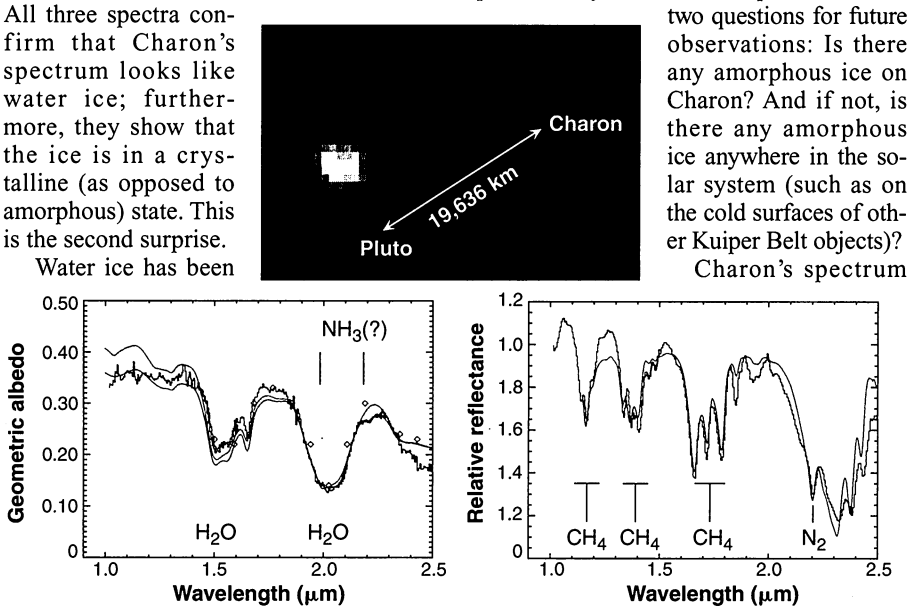
Pluto and Charon are not particularly large (with radii of about 2370 and 1252 km, respectively) and are separated by only 19,636 km (one and a half times Earth's circumference) (*3*). Up until last year, the only spectrum of Charon had been obtained over 10 years ago by exploiting a serendipitous orbital geometry. Throughout 1987 and 1988, Charon could be observed from Earth, passing in front or behind Pluto's disk every 6.38 days (Charon's orbital period). A spectrum of Pluto by itself, obtained when Charon was hidden behind Pluto's disk, was subtracted from the combined Pluto-Charon spectrum. The difference yielded a Charon spectrum at sufficient resolution to show a surface dominated by H_2O ice (*4*).

A surface spectrum dominated by water came as a surprise, because nearby Pluto's spectrum is dominated by methane, CO, and N_2 frosts (see the figure). The current consensus is that Charon has lost the relatively volatile N_2, CO, and CH_4 frosts because of its lower escape velocity, leaving water ice (which is about as volatile on Charon as a rock is in your backyard) behind.

Fast forward 10 years to September 1999, when three groups presented distinct Charon spectra at a workshop held at Lowell Observatory in Flagstaff, AZ (*5*). Two of these were obtained with the Near Infrared Camera and Multi Object Spectrometer (NICMOS), an infrared spectrometer on the Hubble Space Telescope, and the third was obtained with the Keck telescope on a particularly calm night (*2*). All three spectra confirm that Charon's spectrum looks like water ice; furthermore, they show that the ice is in a crystalline (as opposed to amorphous) state. This is the second surprise.

Water ice has been found on satellites of Jupiter, Saturn, and Uranus, and it is always crystalline. This is not surprising, because amorphous ice will rapidly crystallize if the temperature is ~120 K or higher (*6*); indeed, the phase transition from amorphous to crystalline is exothermic. But on Charon, where the surface temperature due to the heat from the sun will never exceed 80 K (and is probably tens of degrees colder), the phase transition should not take place. Instead, crystalline ice should gradually become amorphous under the constant bombardment of UV photons and fast protons. This radiation breaks hydrogen bonds in the ice that subsequently reform, but not in their original crystalline positions. The presence of crystalline ice on Charon may mean that Charon's surface is fresh (for example, resulting from recent deposition of H_2O via comets) or that it was recently hotter than the expected temperature range of 35 to 80 K. Alternatively, some process we have so far failed to consider is converting amorphous to crystalline ice. Its presence raises two questions for future observations: Is there any amorphous ice on Charon? And if not, is there any amorphous ice anywhere in the solar system (such as on the cold surfaces of other Kuiper Belt objects)?

Charon's spectrum



Charon reveals its secrets. This image of the Pluto-Charon binary (**top**) was taken in 1994 with the Faint Object Camera on the Hubble Space Telescope. N_2, CH_4, CO, and H_2O have been identified on Pluto (**bottom right**) and crystalline H_2O (and now possibly NH_3) on Charon (**bottom left**).

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