ponents on very-large scale integrated circuit chips to rerouting garbage collection trucks in Grenoble. The network approach proposed more recently by Hopfield and Tank[24] may prove equally valuable for repetitive tasks where it is worthwhile to build special purpose hardware, and genetic algorithms will receive more attention as parallel SIMD (single instruction, multiple data) and MIMD (multiple instruction, multiple data) computers become more widely available.

The recent history of optimization techniques provides a vivid example of how ideas, developed in apparently unrelated fields, can have a great impact in new areas. Not long ago, few would have predicted that the theoretical study of spin-glasses, evolution, and neural network modelling would provide economical routes for travelling salesmen.

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Functional inactivation of genes by dominant negative mutations

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Molecular biologists are increasingly faced with the problem of assigning a function to genes that have been cloned. A new approach to this problem involves the manipulation of the cloned gene to create what are known as 'dominant negative' mutations. These encode mutant polypeptides that when overexpressed disrupt the activity of the wild-type gene. There are many precedents for this kind of behaviour in the literature—some oncogenes might be examples of naturally occurring dominant negative mutations.

AN awe-inspiring array of techniques is now available for the cloning of genes. For example, the cloned gene may be isolated on the basis of its pattern of expression[1], or by its ability to hybridize to heterologous probes or to code for a particular antigen[2]. The nucleotide sequence may be revealing if it leads to identification of the protein product of the gene and to a suggestion of the general class to which the protein belongs—for example, a DNA-binding regulatory protein, a protease, or a protein kinase. In general, however, the problem of identifying the function of the gene—what its protein actually does in a living cell—will remain. The classical approach is to inactivate the gene and see what effects this has. The function of a gene can, in principle, be inactivated at any of several levels. In yeasts and other lower eukaryotes the wild-type gene can itself be disrupted by targeted insertion, but as yet it is not possible to achieve this in higher eukaryotes, which has been a major stumbling block in determining the functions of mammalian genes that contain the 'homeobox' sequences characteristic of the homoeotic genes of *Drosophila.* One approach that has been suggested involves the disruption of gene function at the level of RNA, but as yet this has met with mixed success.

The purpose of this article is to describe a new strategy in which the function of a gene is blocked at the protein level: the cloned gene is altered so that it encodes a mutant product capable of inhibiting the wild-type gene product in a cell, thus causing the cell to be deficient in the function of that gene product. Such a mutation would be 'dominant' because its phenotype is manifested in the presence of the wild-type gene, and as it inactivates the wild-type gene function it is referred to as a 'dominant negative' mutation. Muller[3] referred to mutations of this kind as 'antimorphs'—"antagonistic mutant genes, having an effect actually contrary to that of the gene from which they were derived". A dominant negative mutation is to be contrasted with other types of dominant mutation, such as those that increase the activity of the gene product—'hypermorphs' in Muller's terminology. Before describing the new strategy I shall review other methods currently in use to inactivate genes in eukaryotes, as a basis for a comparison of the different strategies.

Current strategies

Gene disruption. In some organisms a wild-type gene can be replaced by a mutant version by homologous recombination. The principal virtue of this method is that the gene function is completely destroyed, but at present the technique is only applicable to yeasts[4-6], *Aspergillus*[7,8] and *Dictyostelium*[44]. In higher

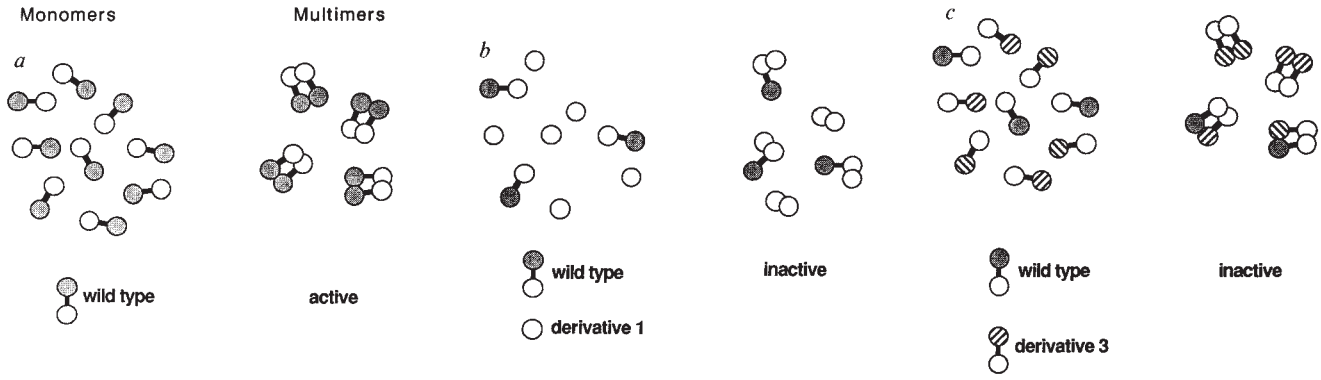

Fig. 1 Inhibitory polypeptides that interfere with the function of multimeric proteins. The polypeptide chain shown here contains two domains, amino-terminal (open) and carboxy-terminal (shaded). *a*, Wild-type polypeptides associate through their amino-terminal domains (as does, for example, the *trp* repressor; see text) to form an active dimeric protein. *b*, Derivative 1 codes for the amino-terminal domain and is able to associate with wild-type chains. When derivative 1 polypeptides are produced in excess, only inactive multimers are formed. *c*, Derivative 3 contains multiple alterations in its carboxy-terminal domain. Association of such monomers with wild-type subunits also leads to formation of dimers that are functionally inactive. Formation of derivatives 1 and 3 is described in Fig. 3 and in the text.

eukaryotes, DNA segments can be added to the genome in random positions, but it is not possible to inactivate the equivalent wild-type gene by targeted homologous recombination. Some examples of homologous recombination involving exogenous DNA introduced into mammalian tissue culture cells have been reported[9,10], but their occurrence is rare.

Antisense RNA. An alternative approach that has attracted much attention involves the use of 'antisense' RNA to block the expression of a gene by preventing the translation of its sense transcripts[11,12]. There have been some notable successes with this method of producing 'mutants without mutations'[13]: for example, a phenocopy of *Drosophila Krüppel* mutants has been produced by injecting antisense *Krüppel* RNA into wild-type embryos[14], a discoidin-deficient slime-mould results from expression of antisense discoidin RNA *in vivo*[15], and antisense RNA of the proto-oncogene c-*fos* can inhibit the platelet-derived growth factor (PDGF)-induced growth of mouse 3T3 cells that express it[16]. Although further successes may result from refinements of this method[12,17–19], the antisense approach has not yet led to the flood of information that I, for one, expected would result from its use.

Imbalance. Meeks-Wagner and Hartwell[20] have reported the creation of a mutant phenotype by overexpression of a wild-type gene. The rationale is that an imbalance of subunit concentration can have dire consequences for the proper formation of multiprotein structures, such as the cytoskeleton and the histone scaffolding of eukaryotic chromosomes. In the particular example described, an altered ratio of histones H2A–H2B to H3-H4 resulting from overexpressing the genes for histones H2A-H2B was apparently the cause of chromosome loss.

Antibodies. Gene function can also be inhibited by the use of antibodies against synthetic antigens based on the predicted sequence of the gene product. For example, antibodies against microtubule components can alter the morphology of intermediate filaments[21,22], and antibodies against *ras* proteins can transiently reverse the transformed phenotype of *ras*-transformed cells[23,24]. Although suitable for the analysis of individual cells or even groups of cells, this method is limited to transient perturbation of the wild-type gene function because of antibody dilution and degradation.

Dominant negative mutations

This new approach involves the inhibition of the function of a wild-type gene product by an overproduced inhibitory variant of the same product. Such inhibitory variants of a wild-type product can be designed because proteins have multiple functional sites that can be mutated independently: for example, sites for oligomerization, substrate-binding, catalysis, membrane

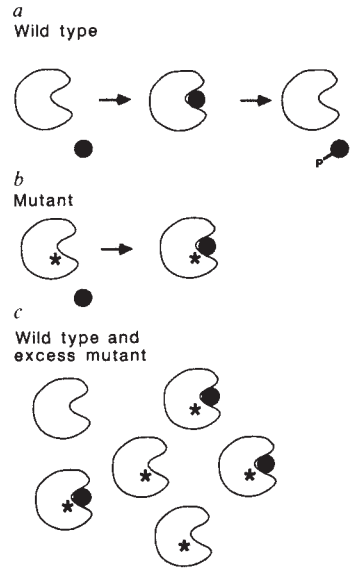

Fig. 2 Inhibitory polypeptides that interfere with function of monomeric proteins. *a*, The enzyme shown is a monomeric enzyme, for example, a protein kinase, which phosphorylates its substrate (black circle). *b*, The mutant form of the enzyme contains an amino-acid substitution that inactivates the catalytic site of the enzyme (asterisk) but that does not affect binding of substrate. *c*, The mutant enzyme acts as a competitive inhibitor of the wild-type enzyme under conditions when the mutant enzyme is overproduced and when substrate is limiting. For example, overproduction of a defective form of the tyrosine kinase coded by proto-oncogene c-*src* might result in a cell depleted for this kinase activity.

association, and so on. Thus, if a protein is multimeric, a derivative capable of interacting with wild-type polypeptide chains but otherwise defective will be inhibitory if it causes the formation of non-functional multimers (Fig. 1). It may also be possible to form inhibitory derivatives of monomeric proteins: if, for example, the activity of a protein is limited by the availability of substrate, then a variant capable of binding substrate but not of carrying out a subsequent catalytic step could be inhibitory (Fig. 2).

In general, therefore, dominant negative mutant proteins will retain an intact, functional subset of the domains of the parent, wild-type protein, but have the complement of this subset either missing or altered so as to be non-functional. It cannot, of course, be known in advance precisely how such a mutant can be created starting with an open reading frame obtained from

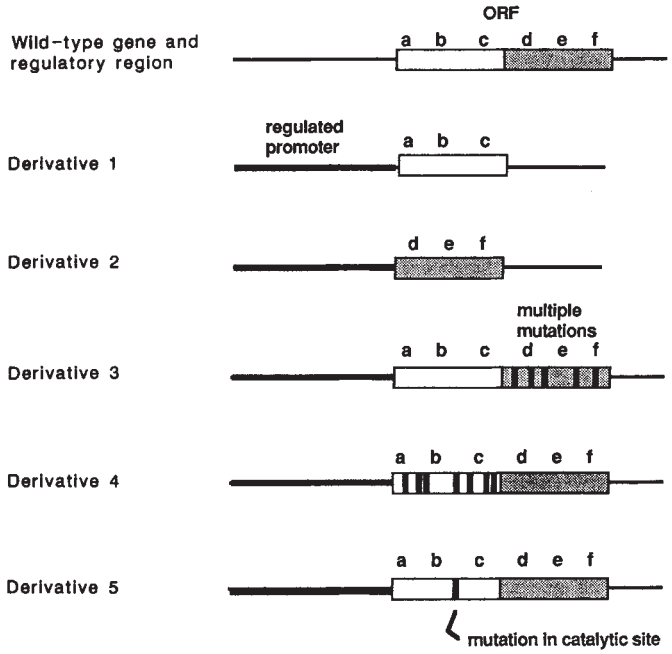

Fig. 3 Structure of a wild-type gene and derivatives designed to overproduce an inhibitory polypeptide. The top line shows the structure of a cloned segment containing an open reading frame (ORF) designated a–f which is read rightwards. The five derivatives all contain a regulatory region upstream of the ORF that confers regulated overproduction of the modified polypeptide coded by the ORF. Transcription is activated, for example, by the addition of steroid hormone or metal ion for the corresponding regulatory regions, or can be governed by tissue-specific regulatory regions. The five derivatives contain alterations in the ORF with the hope that at least one of these modified polypeptides will be capable of interfering with the function of wild-type subunits already in the cell (see Figs 1 and 2). Derivative 1 codes for an amino-terminal segment of the wild-type polypeptide; derivative 2 codes for a carboxy-terminal segment of the wild-type polypeptide; derivatives 3 and 4 code for polypeptides with several amino-acid substitutions in the carboxy- or amino-terminal segments as indicated. If something is known about the function of the polypeptide, an 'educated' derivative (derivative 5) can be designed. For example, the one shown here codes for a polypeptide chain altered for an amino-acid residue that is essential for catalytic activity (as in Fig. 2).

the cloned gene under investigation. However, I suggest that the following four derivatives would give a good chance of creating at least one dominant negative polypeptide (see Fig. 3): derivative 1, which encodes an amino-terminal segment of the polypeptide; derivative 2, which encodes a carboxy-terminal segment of the polypeptide; derivative 3, which is a full-length polypeptide, but with an extensively mutagenized carboxy-terminal segment, and derivative 4, which is analogous to derivative 3, but with the amino-terminus mutagenized. If the enzymatic function of the gene product is known or can be inferred then it might be possible to make specific mutations to generate an additional derivative (derivative 5).

The expression of these derivatives would be put under the control of a regulated promoter, such as the glucocorticoid response element (GRE) of mouse mammary tumour virus, which would confer inducibility by glucocorticoids[25] or a tissue-specific regulatory element[26]. This would have the important consequence that the mutant phenotype would be conditional, being exhibited only when expression of the gene is induced. This would allow propagation of the mutant in the absence of expression of the mutant phenotype, so that the inducible dominant negative mutations can be used in a similar way to temperature-sensitive mutations, for example to define intermediates and steps in some process or pathway. Furthermore, as the mutation is dominant and the phenotype is exhibited in the presence of functional wild-type genes, the consequences of inactivating the function of genes present in multiple copies can be studied without having to inactivate each copy of the gene.

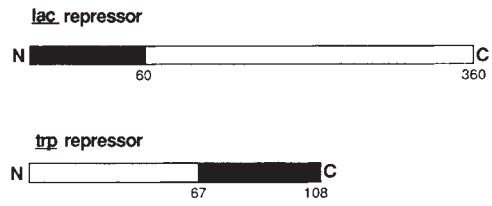

Fig. 4 Structure of *lac* repressor and *trp* repressor polypeptide chains. The structures of *lac* repressor (360 amino acids) and *trp* repressor (108 amino acids) monomers are shown (not drawn to physical scale). The filled regions contain the DNA binding domain, and the open regions contain the oligomerization domain.

How it might work

The efficacy of this approach depends on the likelihood of producing inhibitory polypeptides of the types described above. There are, in fact, a number of precedents for the kind of inhibitory interaction invoked here. In this section I shall explain how these precedents illustrate how the dominant negative mutation approach might work.

Gene-regulatory proteins. Naturally occurring dominant negative mutations affecting the multimeric repressor proteins for the *lac* and *trp* operons, and the lambda repressor have been identified, demonstrating that it is possible to inactivate the DNA-binding domain of such proteins without affecting the oligomerization domain. For example, many amino-acid substitutions in the DNA-binding domain of the *lac* repressor (Fig. 4) create subunits that inhibit wild-type repressor activity when overproduced (reviewed in ref. 27). The oligomerization domain of the *lac* repressor is apparently distinct from its DNA-binding domain, so that mixed aggregates containing mutant and wild-type subunits can be formed and are unable to bind DNA. A dominant negative version of the *trp* repressor can be produced simply by removing its carboxy-terminus[28] (Fig. 4), which generates a polypeptide of the form of derivative 1 (Fig. 3). Cloned genes whose *in vivo* function is not known might contain sequence motifs characteristic of DNA-binding proteins, for example, the 'helix-turn-helix' motif[29,30], which would provide a guide for tailoring gene derivatives of type 5.

There is a second way that inhibitory derivatives of DNA-binding proteins can be created: mutations could turn a transcriptional activator into a repressor. This could occur if the mutation left the DNA-binding domain intact, but destroyed the activity of the transcriptional activation domain. As an example of this, derivatives of the yeast GCN4 protein lacking the domain necessary for transcriptional activation block the function of the wild-type protein[31]. Similarly, mutations of the transcriptional activator encoded by the *x* gene of the human T-cell leukaemia virus HTLV-II have been identified that block the function of the wild-type protein[32]. It should be noted that it is not necessary in principle for a DNA-binding protein to work as a multimer for this strategy to be applicable—inhibitory versions of a protein that works as a monomer might be produced if they bound to the sites recognized by the wild-type protein but did not carry out the requisite activity.

Structures and machines. Proteins that make up structures such as muscle or the mitotic apparatus, or that are involved in cellular morphology appear exquisitely sensitive to defects in monomeric components. For example, mutations in the major actin gene of the nematode have dominant effects on movement[33]. Microtubule function in *Drosophila* sperm is hypersensitive to the presence of defective subunits[34], as is body-shape in the nematode[35,36]. The incorporation of inactive polypeptide subunits into a multiprotein aggregate such as the 'machines' thought to carry out DNA replication and recombination[37]

would be expected to disrupt their function in a dominant manner.

Enzymes. Studies of hybrids formed between wild-type and mutant subunits of the enzyme aspartate transcarbamoylase (ATCase) of *Escherichia coli* illustrate two different ways in which mixed aggregates can have defects in enzyme function. Firstly, mutant subunits altered in active-site residues can associate with wild-type subunits to form non-functional aggregates[38], apparently because the active site of the enzyme is composed of residues from adjacent subunits[39]. Secondly, subunits with changes outside the active site can also inhibit enzyme function in a dominant manner: mixed aggregates fail to respond to binding of a ligand apparently because the protein is 'frozen' in an inactive conformation[40]. Enzymes with many subunits, such as glutamine synthetase with 12 (refs 41 and 42), would be expected to be particularly sensitive to mutant subunits.

Interpretation and inference

As with any genetic method, the use of dominant negative mutant derivatives to learn about gene and protein function requires interpretation and inference. Thus, although the approach is designed to disrupt a wild-type gene function, the overproduction of an inactive product might have the opposite effect—increased activity of the wild-type protein. This might occur if the defective form of the protein titrated a cellular inhibitor. An apparent example of this effect has recently been described for the yeast activator protein GAL4 (ref. 43). A fragment of the catalytic subunit of cyclic AMP-dependent protein kinase might similarly titrate its regulatory (inhibitory) subunit. The overproduction of different derivatives of a protein can thus have very different consequences, some inhibiting protein activity, others enhancing protein activity.

In some cases it is straightforward to determine whether overproduction of the mutant leads to increased or decreased activity of the cellular protein—if the protein in question is an enzyme, for example, in which case assaying enzyme activity would suffice. For a putative regulatory protein of unknown function, however, determining whether the derivative leads to increased or decreased activity of the cellular protein is not necessarily so straightforward, although it is crucial for inferring whether the wild-type protein is an activator or a repressor. Consider, for example, a protein that binds to the regulatory region of a liver-specific gene, and suppose that a mutant derivative of the protein causes hyperexpression of the liver-specific gene. This might mean that the wild-type protein is an activator and that the mutant derivative enhances its activity, as described for GAL4 protein. Alternatively, the wild-type protein might be a repressor and the derivative might be acting as a classical dominant negative mutation. Deciding on which of these possible explanations is the case requires additional analysis.

Concluding remarks

The use of dominant negative mutations could provide information on the *in vivo* function of a diverse array of cloned genes and gene segments. The ability to manipulate genes *in vitro* makes it possible to design inhibitory products based on the principle that the activities of a protein can be separately mutated. The method has the genetic virtues of causing a conditional defect and being dominant, allowing the functional inactivation of redundant genes. Dominant negative mutations may provide a route to new information on protein–protein interactions and hence on designing new inhibitory polypeptides.

Finally, although the focus of this article has been methodological and, in a sense, artificial, the same type of complex mutations discussed in this article might be responsible for some cases of cellular transformation. Both dominant and recessive mutations are known to be able to cause cellular transformation. The dominant mutations in some cases result in hyperactivity of a wild-type product due to overexpression or modification of the product. Recessive mutations, such as that causing retinoblastoma, involve the loss of wild-type function. There is every reason to believe that loss of function could also result from dominant negative mutations. Production of a dominant negative oncogene *in vivo* could involve precisely the same types of events as those described for generating a dominant negative mutation by *in vitro* manipulations. One step would involve modifying the gene product itself to create a potential inhibitory polypeptide. The second step would be an event such as gene amplification that leads to overexpression of this mutant form. The question of whether some oncogenes have resulted from dominant negative mutations is not just a matter of genetic formalism: to understand how the product of an oncogene leads to uncontrolled cell growth, it is crucial to know whether the primary effect of the oncogene is to cause hyperactivity or inhibition of normal cellular activity.

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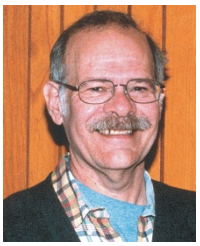

NAOKO ISHIDA

Ira Herskowitz 1946–2003

Anita Sil

On April 28th, 2003, Ira Herskowitz died of pancreatic cancer at the age of 56. As Ira told one of his former students, "Life is not a dress rehearsal." He lived by that philosophy, armed with a passion for biology and legendary clarity of thought.

As the son of geneticist Irwin Herskowitz and the identical twin brother of Joel Herskowitz, perhaps Ira's destiny was intertwined with genetics from the start. After receiving an undergraduate degree in Biology from the California Institute of Technology in 1967, he received a Ph.D. in Microbiology with Ethan Signer at the Massachusetts Institute of Technology in 1971. After a six-month post-doctoral fellowship in the laboratory of David Botstein, Ira moved to the University of Oregon in Eugene to start his own lab at the age of 26. Over the next few years, he expanded his studies of gene regulation in lambda phage to explore mating-type switching and gene silencing in the budding yeast *Saccharomyces cerevisiae*. Each of his classic papers from those early days made striking advances in the understanding of gene regulation and cell-type specification. In the absence of molecular and genomics tools, Ira used simple genetic experiments to reach profound molecular conclusions. These papers are perhaps the best tribute to his logic and precision.

In 1981, Ira left Eugene to join the faculty in the Department of Biochemistry and Biophysics at the University of California, San Francisco, where he ultimately influenced research in many of the surrounding labs. Ira was the head of the Division of Genetics at UCSF for the next twenty years. During that time, his initial work on mating-type switching in yeast continued to branch out into other areas of yeast cell biology. He elucidated the transcriptional circuits that underlie cell-type specification. He studied how yeast cells choose a site of division, how they regulate progression through the cell cycle, how they are able to sense and respond to cells of the opposite mating type and how they regulate meiotic divisions, among other topics. Many of the fundamental discoveries in these fields originated either in his lab or in the labs of his former students and post-doctoral fellows. In more recent years, he became co-director of the Program in Human Genetics at UCSF and helped to launch experiments in pharmacogenomics, the study of how genetic variation influences the response to drugs.

Ira's contributions to biology earned him numerous honors and awards, including a MacArthur Foundation Fellowship (or genius grant), membership in the National Academy of Sciences and, most recently, the prestigious Lewis S. Rosenstiel Award from Brandeis University. His work illuminated the fundamental processes that govern the biology of the eukaryotic cell. Ira lived in fearless pursuit of interesting questions, and his strength was in identifying those questions and using a series of simple experiments to dissect them.

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Even more profound than the research itself was Ira's ability to make its importance clear. He was a master of going to the heart of the matter, whether or not the research in question was his own work. The field of yeast cell biology was deeply influenced by Ira's ability to distill the story behind the results. He made it easy for biologists to appreciate the big picture underlying the data, to understand, for example, how yeast mating-type switching is relevant to asymmetric cell division in higher eukaryotes. After reading Ira's seminal papers and hearing his lectures, many scientists came away with their vision of biology transformed.

Ira had a particular generosity of spirit that made him as passionate about teaching as he was about research. Those of us who had the privilege of hearing his lectures in genetics will never forget the experience of sitting on the edge of our seats, as enthralled by the story he told as we might be by our favorite novel. And no tribute to Ira would be complete without mentioning his irreverent humor, his irrepressible guitar playing and his love of music, all of which went hand in hand when it came to song writing (see http://derisilab.ucsf.edu/movies/returntosender.html). Just as his research chronicled the important events in the life of the cell, his songs commemorated the trials and the joys of life as a scientist. The music of his science, both literal and figurative, will be sorely missed.

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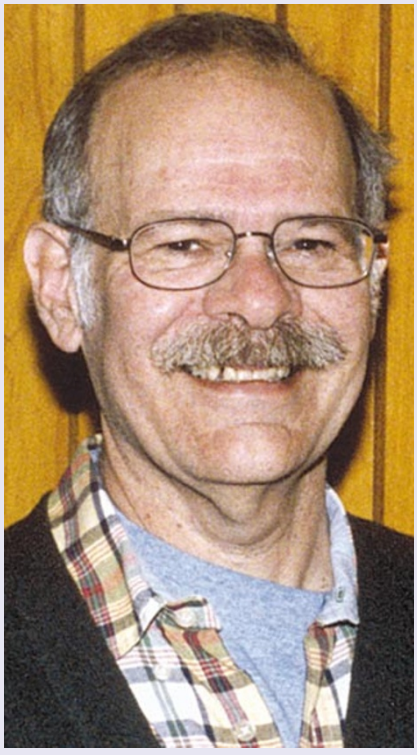
Obituary

Ira Herskowitz (1946–2003)



UNIV. CALIFORNIA, SAN FRANCISCO

Scientist who brought yeast genetics to the forefront of molecular and cell biology

Ira Herskowitz died on 28 April 2003 of pancreatic cancer. He was a scientist of style and influence. Genetics was his path to truth, and he used that approach to make a string of important discoveries in cell and molecular biology.

Herskowitz made an impression early on, as a graduate student with Ethan Signer at the Massachusetts Institute of Technology (MIT) in Cambridge, Massachusetts. He took on the problem of how a protein can activate — increase the transcription of — a gene. Transcriptional activators (as opposed to repressors) were not much in vogue in the late 1960s, and there were no good ideas as to how they might work. Herskowitz studied a protein called Q, which is encoded by the bacterium-infecting virus (bacteriophage) λ and is required for the transcription of a group of other λ genes. He showed that a single DNA site in the λ genome is required for Q to activate all of these target genes. The result revealed that the so-called λ late genes must be transcribed into a single, long messenger RNA, and it set the stage for a full understanding of the molecular workings of Q that is only now coming to fruition.

Thus began a lifelong love affair with bacteriophage λ. Although his lab formally stopped working on the virus in the 1980s, Herskowitz remained engrossed by the subject. In the years before his death he spoke of writing a small book to tell the story of the λ *cII* and *cIII* gene products. Synthesized when λ first enters a host cell, these proteins sense the health of the cell and decide whether the virus should replicate itself and destroy the cell, releasing its progeny in the process, or whether it should silence its genes and integrate into the host genome. The book would have expanded on his influential review on the subject written thirty years ago.

Herskowitz left MIT in 1972 for the University of Oregon, Eugene, where he initiated a series of classic studies on the single-celled yeast *Saccharomyces cerevisiae*. He realized early on (and was instrumental in teaching the rest of us) the power of using yeast as a model organism to study fundamental biological problems. Like humans, other mammals, plants and insects (but unlike bacteria), yeast is a eukaryote — an organism whose cells have defined nuclei. Not only that, but genetic analyses with yeast are about as easy to do as they are with bacteria. This was the opening that fired Herskowitz's imagination, and it was here that he made his greatest impact.

Herskowitz's studies on yeast 'mating-type switching' had a celebrated outcome. *S. cerevisiae* has two mating types, or sexes, and the cells, as they divide, switch back and forth between them. A set of simple experiments confirmed a most unlikely-seeming scenario: programmed DNA rearrangement was responsible for the switching. Few steeped in the world of bacteriophage λ, where proteins bind to DNA to turn genes on and off, would have been prepared to make Herskowitz's leap to a world in which gene control was effected by gene rearrangement.

In 1981, Herskowitz moved to the University of California, San Francisco, where he remained for the rest of his career. There, he initiated a series of studies that revealed fundamental features of eukaryotic cell growth and division. For example, cells often divide asymmetrically, one progeny cell differing in important ways from the other. Herskowitz showed how this occurred in yeast: a key molecular determinant (in this case, a particular mRNA) is distributed to only one of the two progeny cells. The protein produced by this mRNA then initiates a developmental programme that distinguishes one cell from the other. To take another example, every time a yeast cell divides, a molecular mark is left on its surface, thereby recording the history of its cell divisions. Herskowitz showed how the cell then uses these marks to tell which end is which and to establish its subsequent patterns of growth and division.

Herskowitz also explored other aspects of cell biology, including signal transduction, cell-cycle progression, polarized cell growth, chromatin structure, meiosis, gene expression and RNA localization. Virtually all of these studies exploited properties of the yeast mating machinery that he was instrumental in characterizing. Thus, from modest and seemingly specialized questions about the mating behaviour of a single-celled organism, Herskowitz created a body of work with implications for many different branches of molecular and cell biology.

Herskowitz's science was always simply described, yet rich in metaphor and analogy. He trained more than 70 graduate students and postdoctoral fellows. He also helped many more scientists to clarify their ideas, design their experiments and write their papers. A discussion with Ira could entice a biologist, even one who had never studied with him, to delve into new experimental realms. Ira's style of presentation — the simple story, the coloured chalk and the hand-drawn diagrams (where simple arrows inevitably stood in for detailed mechanisms) — became a standard to be emulated. A biological polyglot, he chose his yeast projects with an eye to solving problems under study elsewhere with more complex organisms. He was the master of the simple, telling experiment. He was a gentle man and a gentle scientist. He will remain an unforgettable figure.

Alexander Johnson and Mark Ptashne

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