Epidemiology, Molecular

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Keywords

Biological Markers (Biomarkers)
Biochemical, molecular, genetic, immunologic, or physiologic signals of events in biological systems.

Biologic Markers of Effect
A measurable cellular, biochemical, or molecular alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease.

Biologic Markers of Exposure
A xenobiotic chemical or its metabolite, or the product of an interaction between a chemical, physical, or biologic agent and some target cell or biomolecule.

Biologic Markers of Susceptibility
An inherited or acquired indicator of the response of an individual or a population to a specific xenobiotic agent.

Molecular epidemiology is the use of molecular biological techniques to identify exposures, effects, or susceptibility factors in studies of human populations. Molecular epidemiology and traditional epidemiology utilize the same paradigm. However, the former presents the opportunity to use the enhanced resolving power of molecular biology in the assessment of exposure–disease relationships.

The resolving power, to elucidate a continuum of events between xenobiotic exposure and disease, can provide stronger approaches to research, prevention, and intervention. One particular aspect of the new resolving power of molecular epidemiology is the application of the products of genomic research to epidemiology to assess the genetic components of disease and the interaction between genetic and environmental factors in disease causation. Conversely, the molecular epidemiologic approach may contribute to genomics research by emphasizing the importance of populations and a population perspective. Genetic biomarkers reflect population dynamics and represent useful tools in uncovering complicated interrelationships between environment, culture, and genetics in human history.

1 Principles

The use of molecular biological techniques in epidemiology provides a potentially powerful tool for medical and public health researchers. These techniques allow for the identification of biological markers (Fig. 1) that can indicate exposure to a xenobiotic agent, reveal a biological effect early in the natural history of disease, or represent unique disease subtypes or susceptibility

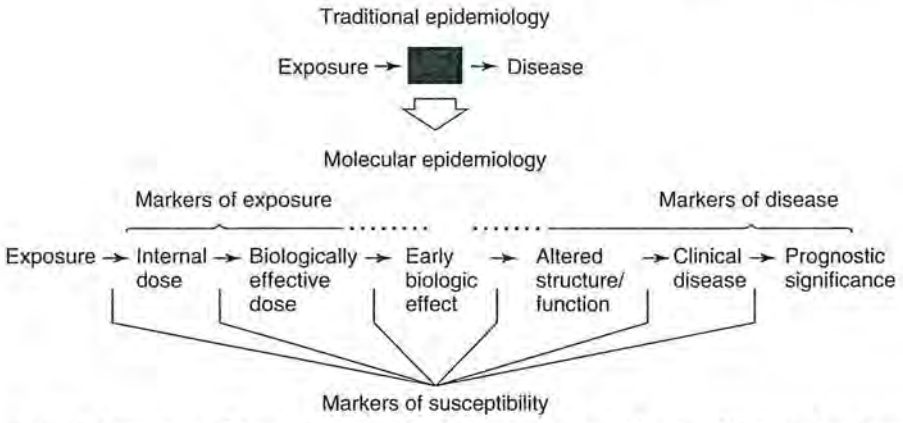

Fig. 1 Enhancement of the traditional epidemiologic paradigm by the use of biological markers resulting in a molecular epidemiologic approach. In traditional epidemiology, the mechanism of action is often a "black box," and associations between an exposure and disease are made by inference. In molecular epidemiology, a continuum between an exposure and a disease is defined, and various markers are identified.

to the development of disease. Although the use of biological markers in epidemiology is not new, the current generation of molecular biological markers enhances past approaches. Epidemiology is an observational science: one makes inferences about disease and health on the basis of comparing groups of people in terms of disease incidence and mortality. Ideally, the groups being compared should be similar in all respects except for the risk factor in question. A benefit of molecular epidemiology is that instead of comparing two groups on the basis of environmental exposure, investigators can compare populations with respect to dose of an environmental agent as measured in critical macromolecules, such as DNA or surrogate proteins for DNA under some conditions, this is presumably a more accurate means of classifying the subjects' true exposure. At the other end of the exposure-disease continuum, instead of using frank disease as an outcome variable in an epidemiologic study, it is possible to use a validated biologic marker of effect.

1.1 Biological Markers of Exposure

The utility of a biological marker of exposure depends, in part, on its half-life, the pattern of the exposure it is measuring (e.g. regular daily exposure vs infrequent episodic exposure), and whether secular trends have occurred in that exposure (e.g. smoking cessation). In addition, the information it provides must be compared with the availability and quality of other sources of data (e.g. questionnaires, environmental monitoring, medical records). Essentially, all exposure measures misclassify some subjects – it is the relative ability of different sources of data to correctly place individuals into exposure categories that must be considered. For example, subjects can generally report average smoking habits and smoking duration in an accurate manner, permitting the calculation of cumulative exposure. Since internal dose markers associated with tobacco smoke have relatively short half-lives, and thus reflect only recent exposure (e.g. cotinine,

a metabolite of nicotine), they have limited utility by themselves to directly assess a smoking–cancer relationship.

In contrast, it is difficult to obtain accurate information about dietary exposure to aflatoxin by using a questionnaire because exposure is sporadic and is present in a spectrum of different food types. In this instance, even a short-term internal dose marker might classify the long-term exposure status of subjects more accurately than questionnaire data. For example, a nested case–control study conducted in Shanghai, China, demonstrated that aflatoxin exposure assessed by measuring several aflatoxin metabolites (and an N_7-guanine aflatoxin adduct) in banked urine samples was associated with an increased risk of hepatocellular carcinoma, while aflatoxin exposure assessed by questionnaire was not associated with elevated risk.

1.2 Biologic Markers of Effect

In terms of disease, techniques are available for identifying biological changes earlier in the continuum between homeostatic response to pathological agents or conditions and development of frank disease. This advance has implications for identifying opportunities for prevention. Biologic markers intermediate between exposure and disease, if validated for disease or risk of disease, can be used to screen people or to allow for early disease detection. These markers can also be used in intervention trials as outcome indicators rather than waiting for subjects to develop disease. There is a popular notion that molecular epidemiology has the potential to contribute to assessment of risk of an individual. However, that point needs clarification. Historically, it has been possible to use an epidemiologic data set, that is, data on a group consisting of sick and healthy people, with and without certain risk factors, to develop risk functions that provide for an estimate of individual risk. This was accomplished in the 1960s using data from the Framingham Longitudinal Study of cardiovascular disease. On the basis of the knowledge of the risk for people with a certain aggregation of characteristics, it was possible to predict the risk for an individual. Molecular epidemiologic approaches provide a means for more confident estimation because more mechanistic information can be utilized. Still, the resultant assessment is only probabilistic determination, not deterministic. While the prediction is that a certain individual is likely to develop disease in the future, there is no guarantee that he or she will develop it. A biomarker may have the utility to screen populations at high risk of disease as part of a primary or secondary prevention effort. However, a substantial amount of information is required before a biomarker can be used for this purpose. In particular, the cumulative probability that an individual will develop disease over a defined period, given a constellation of biologic and nonbiologic risk factors, must be estimated along with a calculation of its uncertainty.

1.3 Biologic Markers of Susceptibility

One of the greatest contributions of molecular epidemiology is the ability to discern the role of host factors, particularly genetic factors, in accounting for variation in response. Why similarly exposed people do not get the same diseases is a target question for molecular epidemiology. In most disease systems, susceptibility markers are being identified and evaluated. These markers can be incorporated

into epidemiologic models as modifiers of the relationship between an exposure and an effect (see Sect. 2.1.3). Traditional epidemiologic approaches can also be enhanced by using molecular genetic techniques to identify host factors that could account for differences in disease risk. Thus, for example, a metabolic polymorphism can be detected from peripheral blood lymphocyte DNA, and groups at potentially greater and lesser risk of disease can be distinguished. The category of markers of susceptibility includes polymorphisms in genes responsible for chemical activation or detoxification, DNA repair, and genomic stability. Susceptibility genes of some types may interact with chemical exposures of very specific types (e.g. cytochrome P450 enzyme subtypes and phase II conjugating enzymes), while others may confer more general susceptibility (e.g. p53 mutations in Li–Fraumeni syndrome). Markers can be measured at the DNA level, if the genetic basis of a polymorphic phenotype has been identified, or at the phenotypic level (e.g. drug probes of hepatic enzyme activity, DNA repair measured in peripheral lymphocytes).

1.4 Utility of Molecular Epidemiology

Molecular epidemiology is a useful temporary blanket term that reflects the reality that increasingly disease, causal exposures, and risk factors are being defined at the molecular level. The utility of this term is that it serves as a signpost for epidemiologists to consider using independent (risk factor) and dependent (outcome) variables that are derived from molecular biological techniques and assays. So, for example, DNA adducts may be used in addition to breathing-zone measurement of a carcinogen, gene mutational patterns may be used as an indicator of disease rather than a nosological death certificate, and genotyping based on the polymerase chain reaction can be used in addition to race and sex to stratify populations for comparisons. In summary, molecular epidemiology has the potential to contribute the following opportunities and capabilities to public health:

- Delineation of a continuum of events between an exposure and a resultant disease
- Identification of exposures to smaller amounts of xenobiotics and enhanced dose reconstruction
- Identification of events earlier in the natural history of clinical diseases and on a smaller scale
- Reduction of misclassification of dependent and independent variables
- Indication of mechanisms by which an exposure and a disease are related
- Better accounting for variability and effect modification
- Enhanced individual and group risk assessments

2 Techniques

If molecular biological markers in epidemiology and public health can be used in the ways described in Sect. 1, they must be demonstrated to be valid in terms of both the assay and the marker. An assay will be valid if it measures what it is expected to measure. A marker will be valid for epidemiologic purposes in two ways. First, it will be valid to the extent that it represents exposure, disease, or susceptibility. Second, it will be valid insofar as the extent of variation in groups with different demographic, behavioral, or medical characteristics is known. A key question

is the prevalence of a particular biomarker (e.g. a mutation) in different ethnic or racial groups, in smokers or drinkers, or in people with various hereditary and acquired diseases. Molecular epidemiologic approaches can be useful in the validation of molecular biological markers in these two ways.

2.1 Representational Validity of Molecular Biological Markers

2.1.1 Validation of the Relationship between Exposure and Dose

Exposure → Internal dose

→ Biologically effective dose

Molecular markers of exposure may be validated by assessing the relation of an exogenous exposure to an internal dose or a biologically effective dose. Critical in validation studies is the need to have an effective exposure assessment. It may be necessary to use a combination of personal and environmental monitoring and questionnaires, record review, and modeling to reconstruct exposure history. The approach also requires an understanding of the pharmacokinetics associated with the particular xenobiotics. Related to this is the need to understand the natural history of the marker and to use it in the validation study. For example, in a study of hydroxyethyl hemoglobin adducts in workers exposed to ethylene oxide, the life span of the erythrocyte, hence the constituent hemoglobin molecule (~4 months), was used as a dosimeter of cumulative exposure. There is also a need to account for factors that might influence the appearance of a molecular biological marker. In the aforementioned studies, when mean values were adjusted for important covariants such as age, cigarette smoking, and education, an exposure–response relationship was found at levels below the permissible exposure level.

2.1.2 Validation of the Relationship between Biological Effects and Disease

Early biologic effect → Altered structure/

function → Clinical disease

Validation information in the biological effects–disease category is limited. The often-repeated question is, "What do the data concerning health and disease mean?" Validation studies of these types are difficult to accomplish because of the temporal factor. Identification of an early effect – that is, an effect in pathogenesis or an effect predictive of disease – generally requires a prospective study design, although cross-sectional clinical studies of diseased and heavily exposed individuals can be used to great advantage. When a prospective design is not used, however, care must be taken to avoid biased associations. This is often difficult; hence prospective studies are the best approach for validation. Prospective studies are expensive and time-consuming, and few are conducted. For example, despite the large number of studies on most of the cytogenetic markers, there is little consensus on their predictive value because most of the studies have been cross-sectional and markers were not linked to disease. Specifically, in epidemiologic terms, predictive value is evaluated in terms of the percentage of those who test positive for a marker and actually develop the disease. Performing the appropriate prospective studies of sister-chromatid exchanges would take a large population and a relatively long time. The best example of such a study is the Nordic prospective study on the relationship between

peripheral lymphocyte chromosome damage and cancer morbidity in occupational groups. Ten laboratories in four Nordic countries participated in a study of a combined cohort of persons (mostly from occupational groups) who had been cytogenetically tested. The cohort is being followed prospectively for cancer morbidity. The cohort comprises 3190 subjects, of whom 1986 subjects (62%) have been scored for chromosome aberrations and 2024 subjects (63%) scored for sister-chromatid exchanges. Preliminary analysis indicates that chromosomal aberrations are associated with cancer. These biologic markers in peripheral lymphocytes represent carcinogenic changes elsewhere in the body. This is the critical criterion of a useful biologic marker. To serve as a valid outcome measure, an intermediate marker must be correlated with disease risk. The criteria for validating intermediate biomarkers have been extensively discussed and include the sensitivity of the marker (i.e. the proportion of subjects who develop cancer and who are positive for the biomarker), the relative risk (a measure of the strength) of the association between the marker and disease, and a judgment about the extent to which the exposure–disease relationship is mediated through a process reflected directly or indirectly by the marker.

2.1.3 Validation of Markers of Susceptibility

Exposure → Susceptibility → Disease

The tools of molecular biology and analytical chemistry have allowed researchers to identify a degree of interindividual variability not previously imagined. Validated biological markers of susceptibility can serve as effect modifiers in epidemiologic studies. Effect modification is a term with statistical and biological aspects. Statistically, the examination of the joint effects of two or more reactors is often discussed in the context of effect modification. It depends on the statistical method (e.g. multiplicative or additive) used to model interaction. From the biological perspective, effect modification contributes to answering the question of why all similarly exposed individuals do not develop a disease. The answer, in part, lies in individual variability in metabolic and detoxification capabilities, their ability to repair genetic damage, or other host factors.

To validate a susceptibility marker, it is important to minimize misclassification, which can occur as a result of laboratory or epidemiologic factors that affect phenotyping or genotyping. Next, it is necessary to demonstrate that the marker increases the risk of disease. The issue of the correlation of acetylation phenotype and bladder cancer from aromatic amines illustrates the concept of susceptibility. Some aromatic amines are detoxified by the enzyme *N*-acetyltransferase, and the slow phenotype of this enzyme has been associated with bladder cancer in exposed individuals. Despite a plethora of studies, the scientific literature is not conclusive on the extent to which being a slow acetylator modifies the risk for bladder cancer in people exposed to aromatic amines. Generally, most studies have been too small, have had weak exposure characterization, and were not designed to allow proper determination of whether exposure or susceptibility was the key factor. An example of how partial validation of a susceptibility marker might occur without using disease as the outcome involved the formation of hemoglobin adducts (which are documented surrogates for DNA adducts

believed to be involved in carcinogenesis) in slow and fast acetylators who had been exposed to 4-aminobiphenyl. Slow acetylators had an average of 1.5-fold greater frequency of adducts than the fast acetylators. Despite these encouraging efforts at validation, few markers of susceptibility have been validated and none are ready for use in population screening.

2.2 Validation of the Behavior of Molecular Biological Markers

Before biomarkers can be used for etiological and prevention research, they need to be validated in populations. This calls for the development of analytical methods for use in large-scale populations. Currently, there is inadequate research support for scaling-up efforts needed for population studies. The validation and scaling efforts discussed here require close collaboration between laboratory scientists and population scientists (clinicians, epidemiologists, industrial hygienists, and exposure assessors). Transitional studies bridge the gap between the development of molecular markers in the laboratory and their application in population-based studies. These studies generally involve the initial evaluation and application of biomarkers in healthy human populations. Their objective is to address issues in sample processing, evaluate assay accuracy and precision, collect information about potential confounders and effect modifiers, and study early biologic effects of selected exposure. Transitional studies can be divided into three broad categories to clarify their distinctive research goals: developmental, characterization, and applied studies. In practice, however, elements of all three types of study are often incorporated into a single field investigation.

2.2.1 Developmental/Characterization Studies

Identification of a promising new molecular biomarker in the laboratory does not mean that the biomarker is ready for use in an epidemiologic study. Other basic issues need to be resolved before its application in human studies can be considered. First, the reliability (i.e. the repeatability of the assay) of a marker must be determined. As long as an assay is reliable, the ordering of subjects by the measure is preserved. Since this is all that is required for studying a marker–disease relationship, reliability, and not accuracy, is of initial importance. Reliability of laboratory assays may be assessed by the analysis of blind replicate samples representative of the range of values likely to be found in human populations. After the assay reliability and accuracy has been determined, it is important to define the optimal conditions for collecting, processing, and storing biological specimens for eventual assay, since, not uncommonly, small variations in these conditions determine the subsequent analyzability of samples. These studies are generally designed to address questions about the presence or the levels of a newly developed marker in the general population. In addition, they serve to identify factors that are confounders or effect modifiers of a marker (e.g. age, gender, medications) that need to be measured and taken into account when applying the marker in subsequent studies.

2.2.2 Applied Studies

Applied studies are investigations performed on subjects with particular patterns of exposure to xenobiotics (e.g.

occupational exposures, smokers) or on patients receiving chemotherapy. In these studies, the biomarker is treated as the outcome variable. At this stage of research, the biomarker has not been shown to predict an increase in risk of disease. The marker, however, can often be used to provide insight into the association between external exposure and biologic processes early in the exposure–disease relationship. Applied studies can help establish the biologic plausibility of associations detected in etiologic studies. Applied studies generally cannot establish a causal relationship between a given exposure, or a given level of exposure, and the risk for developing disease. The results of applied studies using the biomarker as outcome are suggestive only until a marker is shown to predict disease risk, which can be established only by comparing risk of disease in individuals with and without the marker. In these studies, the biomarker may be overly sensitive (i.e. it may respond to low levels of chemical exposures without biological relevance), it may be insensitive, or it may reflect phenomena that are irrelevant to the disease process. Until these relationships have been sorted out, the findings are merely suggestive.

2.3 Etiologic Studies

The major objective of molecular epidemiology is to conduct etiologic and applied research. Etiologic studies [i.e. ecologic, case–control, case–case (also referred to as a case series), prospective cohort, family, twin, and intervention studies] can be distinguished from transitional studies in that the former involve either clinically ill subjects, asymptomatic subjects with early disease, or subjects positive for an intermediate process known to be associated with increased risk of disease (e.g. colon adenomas and risk of colon cancer). Case–control, case–case, and prospective etiologic studies can effectively utilize molecular epidemiologic approaches.

2.3.1 Case–control Studies

A case–control study involves the comparison of cases (people with a particular disease) with controls (people without that disease) for various risk factors. The risk factors could be an exposure, a trait, or a biomarker. Traditionally, case–control studies have involved patients with clinically confirmed disease, identified either through the presence of symptoms or as a result of incidental findings on routine clinical examinations. Increasingly, cases are being defined as asymptomatic subjects whose early preclinical disease has been ascertained by screening (e.g. early breast disease, colon polyps, cervical dysplasia). The case–control study design is used far more frequently than the prospective cohort study design because of its relatively greater efficiency and lower cost. Therefore, maximizing opportunities to creatively integrate biomarkers into case–control studies is important. Because some markers are affected by disease itself, which raises complications of reverse causality, it is important to define which biomarker categories can most effectively be used in this study design.

2.3.2 Case–case Studies

A case–case study involves a series of cases of the same type that are compared on the basis of a particular exposure and a particular biologic characteristic. The accumulation of a spectrum of *ras* oncogene mutations in leukemia cases with benzene exposure compared with those in

leukemia cases without benzene exposure is an example of a case–case study. Such studies have the potential of identifying an exposure-specific effect at the molecular level. Case–case studies, however, cannot be used to estimate the relative risk of disease from a specific exposure. A nondisease control group is required for this purpose. Case–case studies offer great precision in investigating gene–environment interactions when the disease outcome of interest is rare.

2.3.3 Prospective Cohort Studies

Prospective studies involve healthy people, characterized by the presence or the absence of a risk factor, who are followed forward to determine the risk of disease. In prospective studies, the biological samples may be collected from subjects at various times. These samples are either analyzed at the time of collection or banked for later analysis. One way to utilize biologic markers in a prospective study is to follow the groups of subjects forward in time: subjects who develop disease are identified, and premorbid biomarker levels in the group with disease are compared with those without disease. Often, a nested case–control approach is used. Samples from cases and only a sample of the controls (noncases) are analyzed, which considerably reduces the laboratory requirements and costs. Although the prospective study design is by far the most time-consuming and expensive type of observational epidemiologic study, it is the only method available to test the association of biomarkers with disease risk when the markers are transient or may be directly or indirectly affected by disease. Prospective studies may yield banks of biological specimens that are useful for future studies. Large cohort studies initiated in the 1980s and 1990s are, in general, banking most or all fractions of the peripheral blood sample to allow a far wider range of biologic assays to be performed, particularly those that require DNA.

2.4 Molecular Epidemiology and Genomics

Molecular epidemiology and genomics utilize the products of genomic research to identify variation in any gene considered relevant to disease as well as the "genome-wide" genotype for every participant in a study. Additionally, products of genomics research will allow classification of diseases in new ways, possibly leading to new interventions. Evaluation of gene–environment interaction is becoming an increasingly important topic that will be complicated by large numbers of gene–gene and gene–environment interaction candidates. Larger-sized study populations (in thousands) will be required to study more than a few genes and environment factors.

Molecular epidemiology can contribute to addressing the issue of massive amounts of data that result from genomics research. The reduction, summarization, analysis, and interpretation of data from a population perspective are necessary to distinguish homeostatic profiles from pathologic profiles. Molecularly defined subsets may be the bases for new classification or definitions of disease.

2.5 Public Health Applications

Public health practice incorporates the end use of validated biomarkers for risk assessment by government agencies, for population screening (both active and

passive), and for clinical and preventive medical practice. The use of molecular biomarkers in public health practice is still in its infancy, although several very promising markers may soon find their way into the public health arena. The standard principles of biological monitoring and medical screening are applicable to the use of any biomarker, however. These include assay reliability and cost, strength of the association between a marker and disease risk, prevalence of the marker in the population, availability of effective, preventive strategies that can be employed in subjects who are positive for the marker, and a host of ethical considerations, such as informing subjects of test results.

See also Genetics, Molecular Basis of.

Bibliography

Books and Reviews

Gledhill, B.L., Mauro, F. (Eds.) (1990) *New Horizons in Biological Dosimetry*, Wiley-Liss, New York.

Groopman, J.D., Skipper, P.C. (Eds.) (1991) *Molecular Dosimetry and Human Cancer: Analytical, Epidemiological and Social Considerations*, CRC Press, Boca Raton, FL.

Hulka, B.S. (1991) Epidemiological studies using biological markers: issues for epidemiologists, *Cancer Epidemiol. Biomarkers Prev.* **1**, 13–19.

Hulka, B.S., Wilcosky, T.C., Griffith, J.C. (Eds.) (1990) *Biological Markers in Epidemiology*, Oxford University Press, New York.

Millikan, R. (2002) The changing face of epidemiology in the genomics era, *Epidemiology* **13**, 472–480.

Schulte, P.A. (1993) The use of biological markers in occupational health research and practice, *J. Toxicol. Environ. Health* **40**, 359–366.

Primary Literature

Perera, F.P., Weinstein, I.B. (1982) Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation, *Chronic Dis.* **35**, 581–600.

Rothman, N., Stewart, W.F., Schulte, P.A. (1995) Incorporating biomarkers into cancer epidemiology: a matrix of biomarker and study design categories, *Cancer Epidemiol. Biomarkers Prev.* **4**, 301–312.

Schatzkin, A., Freedman, L.S., Schiffman, M.H., Dawsey, S.M. (1990) Validation of intermediate endpoints in cancer research, *J. Natl. Cancer Inst.* **82**, 1746–1752.

Schulte, P.A., Perera, F.P. (Eds.) (1993) *Molecular Epidemiology Principles and Practices*, Academic Press, San Diego, CA.

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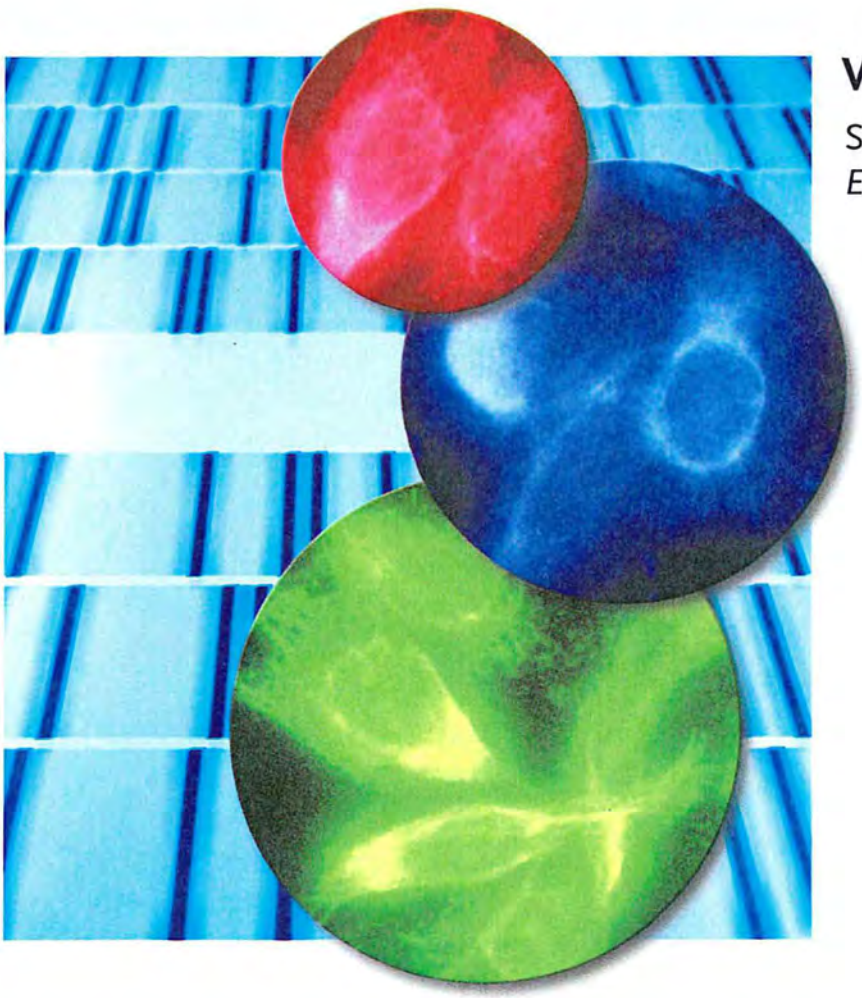

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