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cell types. The excitation and emission properties of drFP583 in the green and the red channels minimize the pesky background fluorescence of cells in the absence of expressed DsRed such that fluorescent timer applications may readily lend themselves to high-throughput ratiometric screening. Tried and true technologies already exist for high-throughput screening of gene reporter activity in cultured cells, so at least initially the DsRed-based reporter is more likely to be used as a secondary screening tool. Eventually, the kinetic attributes and versatility of DsRed-based reporters for other applications discussed below may even permit it to supplant existing primary screens.

Immediate advances may be found in the application of fluorescence timer technology to problems that are more difficult to monitor by existing techniques. The rate of protein degradation also will affect the red:green fluorescence ratio of DsRed (Fig. 1). Protein turnover is an important component of physiological action and drug response that, to date, has been laborious to study. An in-frame fusion of a target protein to the extremely stable DsRed likely will assume the turnover rate of the target protein. Altered stability of the target protein following a physiological or pharmacological stimulus then would be reflected in the altered stability of the fusion protein, which would be measured as an alteration in the red:green fluorescence ratio.

The fluorescence timer also will be useful for determining the temporal sequence of intracellular trafficking. Specific cellular substructures can be correlated by microscopy with the intracellular distributions in the red:green fluorescence emitted from DsRed-linked proteins. A structure displaying more green fluorescence character would be one that is occupied by the linked protein at an earlier time after synthesis than is a structure displaying more red fluorescence character. This will facilitate the identification of new classes of compounds that affect a protein's movement and location, which in many cases is just as important as a protein's amount and isolated activity.

The properties of DsRed and its derivatives are not yet optimal. The propensity of DsRed to form a tetramer[5] is currently one such limiting property that may cause some linked proteins to acquire properties not present in the native protein. Baird *et al.*[5] used this oligomerization to demonstrate that green fluorescence from the immature, green conformer of DsRed is transferred to an adjacent mature red conformer by fluorescence resonance energy transfer (FRET). Therefore, FRET will affect the ratio of red:green fluorescence and complicate measurements of the timer function if FRET changes between measurements. Even once mutants deficient in oligomerization are identified, FRET between immature and mature DsRed brought into close proximity by multimerization of a linked target protein still will need to be measured in parallel and accounted for.

The development of fluorescent timer technology is still in its infancy but offers much promise. A drug or stimulus found in an initial screen to alter the red:green ratio of DsRed may have an effect at any of a diverse set of levels (Fig. 1), from the synthesis of the reporter or fusion protein, to protein degradation, to alterations in the multimeric status of the target protein or DsRed itself (FRET), to even the maturation of DsRed. This breadth of responses may allow the investigator to rapidly identify compounds that affect a variety of events that can be readily discerned in follow-up experiments. The properties and known structures of the many GFP homologs and derivatives identified to date will certainly aid the identification of DsRed derivatives with more optimal qualities[3–6]. These advances, together with existing GFP-based technologies, will allow the noninvasive spatial, temporal, and physical characterization of a wide variety of biochemical events within the context of the living cell. The ability to rapidly measure these effects before and after the addition of a stimulus will make these assays readily amenable to high-throughput screening.

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Aneuploidy and cancer—the vintage wine revisited

Harvey Bialy

Twenty-five years ago, Peter Duesberg isolated and characterized the first viral oncogene—the principal base on which the predominant, current gene mutation theory of cancer rests. During the past five years, Duesberg has been applying a similar determination investigating an alternative genetic explanation of cancer that derives from a century-old observation concerning the chromosomal abnormalities (aneuploidies) associated with essentially every solid human tumor[1]. Now in a paper published in the most recent issue of *PNAS*[2], he and his co-workers have provided compelling evidence that the high mutation rates of cancer cells are due to an aneuploidy-based continuous chromosome reassortment.

In a series of papers between 1997 and 2000, Duesberg and his colleagues at the University of California, Berkeley and the University of Heidelberg at Mannheim showed that aneuploidy, an imbalance in chromosome number, is diagnostic in the earliest stages of cell culture of malignant transformation induced by non-genotoxic carcinogens, such as polycyclic aromatic hydrocarbons (PAHs)[3]; that aneuploidy perfectly explains the notorious genetic instability of cancer cells[4]; and that aneuploidy is a transformation-related event, because it precedes malignant transformation, and because 14 out of 14 cancers (100%) of inbred Chinese hamsters (CH) were aneuploid compared with about 30% of PHA-treated CH cells[5]. (The chance that aneuploidy of the 14 cancers was unrelated to transformation is only 0.30^{14}, or $\sim 5 \times 10^{-8}$.) In addition to these experimental supports, the aneuploidy explanation of cancer was given a rigorous formalization based on the mathematical and logical underpinnings of metabolic control analysis[6], until relatively recently an arcane biochemical discipline that has nonetheless replaced the textbook concept of a rate-limiting enzyme with the real-world idea of distributed control[7].

But the best tests of any theory are its accuracy at making experimental predictions in settings not directly related to those in which it was formulated, and its ability to explain previously inexplicable findings. In the latest *PNAS* paper[2], aneuploidy as the functional genetic basis of cancer passes both these tests in ways that should be of interest to biotechnologists of a variety of stripes.

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Of all the peculiarities of cancer cells, the extraordinarily high rate at which they become resistant to chemotoxic agents (one in one thousand to one in one million per mitosis) has for almost 50 years remained one of the more difficult for theoreticians to explain and clinicians to confront. As recently as 1995, Henry Harris considered it to be a "major conceptual difficulty to reconcile the very high mutational frequency with genetic theory if two functional alleles are present in the same cells."[8] In light of this, numerous investigators have proposed that "epigenetic mechanisms" might be responsible for drug and multidrug resistance in cancer cells, although before now no specific mechanism had been suggested.

Because aneuploidy simultaneously imbalances, through effects in gene dosage, large numbers of balance-sensitive proteins, including those involved in the mitotic spindle apparatus, its occurrence in a cell results in a self-perpetuating chromosomal instability. The average cell in a typically aneuploid tumor, for example, is at a 46% risk to gain or lose one chromosome per mitosis[3]. Continuous chromosome reassortment, catalyzed by aneuploidy, as Duesberg and his colleagues argue in their most recent paper, is a likely mechanism to explain the high mutation rates of cancer cells. It may reflect more than simple coincidence that this model has an exact precedent in the mechanistic explanation of the influenza virus' exceptionally high mutation rate through reassortment of subgenomic RNA segments that Duesberg provided in the pages of a 1968 number of the *PNAS*[9].

In the present work, the idea of aneuploidy-catalyzed reassortment as the epigenetic "mutator" is directly tested by comparing the mutation rates of aneuploid, tumorigenic cells with those of diploid, normal cells from the same inbred line of Chinese hamsters. As all of the cells studied have an otherwise identical genetic background, any differences in mutation rates must result from their different chromosome numbers. The mutations investigated were to resistance against the anticancer drugs puromycin, cytosine arabinoside, colcemid, and methotrexate. Exactly in accord with a chromosomal reassortment model, the mutation frequencies of aneuploid cells were high, between 10^{-4} and 10^{-6}, whereas the frequency of resistance in the diploid cells was undetectable.

In addition to the quantitative dilemma that cancer cells pose for gene mutation theories by their high mutation rates to single-drug resistance, it is completely bewildering to these theories that cancer cells often become simultaneously resistant to a number of functionally and structurally unrelated drugs[10]. But such a result is precisely what one would expect if a chromosomal reassortment model were applicable, as large numbers of genes are being affected simultaneously. Experimentally, this means that selection for one phenotype should produce cells possessing novel, unselected phenotypes. As succinctly put in the paper's abstract, "Mutants selected from cloned (aneuploid) cells for resistance against one drug displayed different unselected phenotypes, e.g., polygonal or fusiform cellular morphology, flat or three-dimensional colonies, and resistances against other unrelated drugs."

One final point in the support of the reassortment model is that it immediately explains the otherwise puzzling finding that the drug resistance mutations of cancer cells are significantly less stable than conventional mutations, some reverting to the original phenotype at the same rate with which they were generated[10]. The constantly shifting karyotype responsible for the drug resistance can also lead to its loss. Indeed, preliminary evidence for this interpretation is presented by the observation that "in many cultures of drug-resistant cells growing in the presence of a selective cytotoxic drug (there are) an excess of unattached, dead cells, compared with parallel cultures grown in the absence of the drug."

Beyond providing the theoretician with a unifying explanation of the high mutation rates of aneuploid cancer cells, and the frequent occurrence of multidrug resistance, the chromosomal reassortment model offers the more practical minded a potential way to determine effective chemotherapeutic regimes, as it predicts the existence of phenotype-specific karyotypes still to be identified. And perhaps with immediate applicability, it suggests using the aneuploidy-based, high frequency of drug resistance in cancer cells as a functional alternative to direct determinations of aneuploidy in order to diagnose the presence of preneoplastic cells in benign lesions.

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