nical complication that will need to be addressed for longer proteins is that all of the required aminoacyl-tRNAs must be included in the translational mix. Most simply, this could be achieved by adding cellular fractions containing the various translational components. In this case, however, the natural aminoacyl-tRNA for the reassigned codon would have to be prevented from acting, possibly with the use of a specific RS inhibitor.

In a report that was published in the June issue of *Chemistry & Biology*, Dougherty and coworkers [14] electroporated synthetic genes and chemically aminoacylated tRNAs directly into mammalian cells. Electroporation may allow higher transfection yields than other methods, such as biolistics, microinjection, and lipid-based carrier treatments, and it has the added advantage that it may also be applicable to a wide array of cell types. Other techniques, such as microinjection, may only work well with relatively large cells, like oocytes. Moreover, electroporation simultaneously transfects large populations of cells, whereas cells must be microinjected individually. Dougherty and coworkers electroporated both CHO cells and primary neurons. Two synthetic genes were used, one for green fluorescence protein and the other for the nicotinic acetylcholine receptor (nAChR). Each gene was engineered to contain the UAG codon at a single position and the aminoacyl-tRNA containing the appropriate anticodon. The electroporated transfectants expressed the encoded messages and made proteins containing the amino acid donated by the aminoacyl-tRNA. In separate experiments, tRNAs were acylated with either the natural serine or the unnatural 5,7-difluorotryptophan (F2trp), and both amino acids were incorporated.

Importantly, the F2Trp-engineered nAChR confers a characteristic effect on cell physiology. Using an oocyte microinjection system, Dougherty had previously shown that fluorinated tryptophans incorporated at residue 149 decrease the sensitivity of the receptor to acetylcholine [15]. In the current work, they use this phenomenon as an assay to demonstrate F2Trp incorporation in electroporated CHO cells. Thus, this work lends hope that reagent electroporation may greatly facilitate one of the most promising uses of the unnatural amino acid technology: the characterization of protein function in living cells.

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Screening for Inducers of Kinase Degradation

Targeted small molecule-induced protein degradation is a promising approach to inhibit signaling within kinase cascades. In this issue, researchers describe a simple assay for the rapid, high-throughput identification of novel agents that promote degradation of the kinases Her2 and EGFR.

Her2 is a transmembrane receptor tyrosine kinase that heterodimerizes with other members of the Her family (e.g., epidermal growth factor receptor (EGFR)/Her1, Her3, and Her4) and promotes the transduction of proliferative and survival signals [1]. Her2 is overexpressed in a significant proportion of adenocarcinomas, and clin-

ical studies have demonstrated that elevated Her2 expression correlates with poor prognosis in multiple malignancies, including breast and ovarian cancer [2, 3]. The kinase has therefore been identified as a valuable molecular target for the treatment of these cancers.

Expression of EGFR at high levels is also associated with aggressive cancer and a poor clinical prognosis [4, 5]. Normally, epidermal growth factor (EGF) binding to EGFR homodimers stimulates receptor downregulation (via internalization), and this depends on recruitment of the E3 ubiquitin ligase, c-Cbl, to the phosphorylated receptors followed by c-Cbl-mediated EGFR ubiquitination and degradation [6]. In contrast, although certain tumor inhibitory Her2 antibodies, such as Herceptin, enhance recruitment of c-Cbl to Her2, thereby accelerating its internalization and degradation [7], in the absence of such antibodies phosphorylated Her2 only weakly associates with c-Cbl and thus is resistant to c-Cbl-induced downregulation [1]. Indeed, Her2 heterodimerization with EGFR antagonizes association of EGFR with c-Cbl, thus promoting receptor longevity and recycling to the cell surface [8]. For this reason and because point mutations that constitutively activate Her2 kinase activity are rarely found in Her2-overexpressing tumors [9], inhibition of Her2 kinase activity per se might be expected to prove less beneficial than approaches that focus on downregulating expression of either Her2 or EGFR. Thus, identification of novel means to regulate the stability of these kinases should provide additional opportunities for successfully interdicting signaling through Her2- and EGFR-containing receptor complexes.

Within the last few years, some agents capable of promoting Her2 and/or EGFR degradation have been described. For example, we recently reported that stability of mature Her2 requires association with the molecular chaperone hsp90, and hsp90 inhibitory small molecules are able to rapidly destabilize the kinase [10]. Coupled with these data, hsp90 inhibitors have shown promising antitumor activity in animal models of Her2-overexpressing tumors [11]. Further, novel irreversible Her2 inhibitors, such as CI-1033, have been shown to promote Her2 degradation in vitro [12] and to have potent antitumor activity in vivo [13]. Both an hsp90 inhibitor and CI-1033 are currently undergoing clinical trial in cancer patients. Other diverse agents that possess antitumor activity in animal models and which in some cases have been or are currently in clinical trial, including the deacetylase inhibitor FK228 [14], the natural product curcumin [15], and the broad-spectrum kinase inhibitor staurosporine [16], also promote degradation of EGFR and/or Her2. While the chaperone-associating E3 ubiquitin ligase CHIP has been identified as a possible mediator of hsp90-dependent Her2 degradation [17], the mechanism(s) responsible for Her2/EGFR degradation stimulated by FK228, curcumin, and staurosporine remain to be elucidated. The contribution of kinase degradation to the anticancer properties of these varied compounds must be carefully explored, but the data clearly demonstrate that stimulation of Her2 and EGFR degradation is feasible and does correlate with the antitumor activity of several structurally distinct agents.

Thus, combining the specificity of kinase inhibitors with the effectiveness of kinase degradation holds great promise for cancer treatment, but such approaches are currently in their infancy. Previously, identification of compounds that promote Her2 and/or EGFR degradation has required cumbersome in vitro analyses involving tissue culture with individual drugs followed by detergent lysis of samples, polyacrylamide gel electrophoresis of cellular proteins, and Western blotting to determine Her2 and EGFR levels. Identification of new agents that induce Her2 and/or EGFR degradation is clearly warranted and could lead to novel and exciting therapeutic strategies; however, the current methodology is decidedly unsuitable for rapid, high-throughput screening of compound libraries.

The paper in this issue of *Chemistry & Biology* by Chiosis et al. [18] describes a simple, cell-based, microtiter plate assay to quantify Her2 and EGFR protein levels. This assay is readily adaptable to a high-throughput format and thus should allow for the rapid screening of compound libraries to identify novel inducers of Her2 and EGFR protein degradation. The method, which relies on whole-cell immunodetection of the proteins in question, utilizes a minimal number of cells, yet is sufficiently sensitive and reproducible to permit quantitative determinations. The microtiter plate format requires expenditure of minimal amounts of unknown compound, thus making this an ideal platform for small molecule library screening. This fast and reliable assay should greatly improve the speed with which novel Her2 and EGFR degradation inducers can be identified and developed for ultimate clinical use.

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