Reengineering growth factors "through the looking glass"

Thomas L. Ciardelli

Alice: "I'll tell you all my ideas about Looking Glass House. First, there's the room you can see through the glass—that's just the same as our drawing-room, only things go the other way."

Lewis Carroll

Through the Looking Glass

Over the past five years, the road to the discovery of new drugs has taken a remarkable turn. A staggering sequence of technological achievements in molecular biology, chemistry, and engineering has dramatically altered approaches to the identification of new therapeutic agents. More often than not, the quest for a new drug involves an automated, high-speed search through structural databases or through libraries containing thousands to millions of compounds constructed by combinatorial methods. In the majority of these techniques, the meter that identifies candidate molecules ("hits") in a sea of similar structures is binding affinity. Selecting compounds based on the affinity of their interaction with target molecules, followed by amplifying the interaction ("affinity maturation") through covalent alteration has become a major focus of biotechnology. The success of these approaches in revealing structurally novel "binders" is irrefutable and has greatly accelerated the pace of the discovery of lead compounds.

Given these achievements, what possible significance could be associated with the report in this issue by Reddy et al.¹ (pp. 1696–1699) of an epidermal growth factor (EGF) analog having significantly reduced receptor binding affinity? Are these workers carrying out their studies in the Looking Glass House? Quite "contrariwise," the significance of their report is evident from the title—"Engineering Epidermal Growth Factor for Enhanced Mitogenic Potency"—as it

represents a novel approach to ligand design based on potency.

Among the final arbiters of the success of any new drug development program are potency and efficacy, not necessarily affinity. Potency and efficacy are concepts familiar in traditional pharmacology and are intimately linked to the receptor dynamics of the tissue, whereas affinity is a physical chemical constant measured in static assays (see Fig. 1). Should we be surprised to learn that affinity and potency do not necessarily correlate? I hope not. Even the astounding 400-fold enhancement of receptor binding affinity reported for a human growth hormone analog was not associated with increased agonist potency².

To some extent, the pharmacological implications and traditional concepts of receptor theory have become a casualty of

cy of an agonist ligand depends upon the number of receptors that it occupies at a given concentration as well as the duration of occupancy. Any parameter that alters ligand and concentration or receptor density will reflect on potency. Reddy et al. clearly show how ligand affinity may influence both parameters as a result of ligand/receptor trafficking and endosomal sorting. Because of the relative rates of endocytotic process, the potency of the natural growth factors EGF and transforming growth factor- α are attenuated either by swift receptor down-regulation or rapid ligand degradation. By virtue of its lower affinity, however, an analog of EGF carrying a point mutation (Tyr13→Gly) suffers neither fate and is ultimately more potent.

The authors conclude that in an effort to optimize response, cellular trafficking processes controlling the fate of both ligand and receptor are often more important than binding affinity. Indeed, too great an affinity may adversely influence potency because of accelerated degradation of ligand and/or receptors.

Are these findings unique to the properties of the EGF system? Probably not—related observations have been reported for interleukin-2 analogs^{7,8}. Each ligand/receptor system, however, will have different cellular trafficking parameters and it will be very important to examine them carefully, as these authors have done, in order to exploit trafficking dynamics in ligand design.

This report has important implications for those involved in discovering or optimizing growth factor analogs and other ligands for therapeutic and bioprocessing applications. In the pursuit of higher affinity, it is possible that one might be taking the fast track in the wrong direction. Armed with an understanding of ligand/receptor trafficking dynamics, the goal of greater potency may be more readily achieved.

Figure 1. Potency is dependent upon cellular trafficking dynamics as well as affinity.

the rush to discover new drugs. It has been known for decades that the potency and efficacy of a drug are dependent upon intrinsic properties of the molecule as well as on the receptor dynamics of the target tissue³. Engineering of protein and peptide ligands for enhanced affinity is now a comfortable idea. Affinity is an intrinsic property of a ligand that is easily measurable. Is it possible to engineer a protein ligand so as to not only change its affinity, but also to alter the receptor dynamics of the target tissue?

Reddy et al. demonstrate that it is possible. This report is only the latest in a series of detailed studies by these authors^{4,5} that carefully quantify the parameters of ligand/receptor intracellular trafficking processes for the EGF receptor system. In this system (as well as for most growth factors⁶), poten-

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