

# Codominance and toxins: A path to drugs of nearly unlimited selectivity

(diseases/antiviral therapy/cancer/proteolysis/"comtoxin")

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**ABSTRACT** The effectiveness of drugs is often limited by their insufficient selectivity. I propose designs of therapeutic agents that address this problem. The key feature of these reagents, termed comtoxins (*codominance-mediated toxins*), is their ability to utilize codominance, a property characteristic of many signals in proteins, including degradation signals (degrons) and nuclear localization signals. A comtoxin designed to kill cells that express intracellular proteins P1 and P2 but to spare cells that lack P1 and/or P2 is a multidomain fusion containing a cytotoxic domain and two degrons placed within or near two domains P1\* and P2\* that bind, respectively, to P1 and P2. In a cell containing both P1 and P2, these proteins would bind to the P1\* and P2\* domains of the comtoxin and sterically mask the nearby (appropriately positioned) degrons, resulting in a long-lived and therefore toxic drug. By contrast, in a cell lacking P1 and/or P2, at least one of the comtoxin's degrons would be active (unobstructed), yielding a short-lived and therefore nontoxic drug. A comtoxin containing both a degron and a nuclear localization signal can be designed to kill exclusively cells that contain P1 but lack P2. Analogous strategies yield comtoxins sensitive to the presence (or absence) of more than two proteins in a cell. Also considered is a class of comtoxins in which a toxic domain is split by a flexible insert containing binding sites for the target proteins. The potentially unlimited, combinatorial selectivity of comtoxins may help solve the problem of side effects that bedevils present-day therapies, for even nonselective delivery of a comtoxin would not affect cells whose protein "signatures" differ from the targeted one.

Abnormal cells differ from their normal progenitors and other cells of the same organism in a variety of ways, including protein composition. For example, virus-infected cells contain virus-specific proteins; the levels of certain cellular proteins are also altered as a result of viral infection. Cancer cells, which can grow at sites of their initial emergence and at distant sites that they are capable of colonizing, differ from their normal progenitors in the patterns of gene expression. Some of the tumor-specific proteins are altered versions of normal proteins, in that they are encoded by genes whose mutations were among the causes of a malignant phenotype (1–3).

A viral genome encodes relatively few proteins. In part because most of these proteins have functional counterparts in cells that the virus infects, effective antiviral drugs remain, by and large, a goal to be reached (4). With malignant tumors that cannot be eliminated by surgery alone, the problem of finding a drug up to the task is even more complicated, because compositional differences between a tumor cell and its normal progenitor can be subtle and quantitative rather than qualitative. In addition, cells of a tumor are often heterogeneous genetically and in protein composition (1–4). These are some of the reasons for the failure of present-day therapies to cure

most cancers. A major limitation of cytotoxic treatments is their low selectivity for tumor cells. For example, radiation therapy and alkylating agents perturb many functions that are common to most cells. As for the current single-target drugs, for example, methotrexate and vinblastine (4), the problem is not only the distribution of their ligands (dihydrofolate reductase and tubulin), which are present in both normal and malignant cells, but also the constraint that a single molecular target is often insufficient for defining unambiguously enough the cell type to be eliminated.

A recent approach to improving the selectivity of drugs involves the linking of a toxin to an antibody or another ligand (e.g., a growth factor) that binds to a target on the surface of tumor cells. The tumor selectivity of these reagents, called immunotoxins or chimeric toxins, is often higher than that of small cytotoxic drugs (5, 6). Unfortunately, a surface marker that an immunotoxin recognizes may be present not only on target cells. Moreover, since this marker is often not essential for tumorigenicity, there may be cells in a tumor that lack the marker but are still malignant. These are some of the limitations of present-day immunotoxins (5, 6).

Yet another approach is to increase or redirect the power of the immune system to identify and selectively destroy tumor cells. Immunotherapy of cancer has a long and checkered history. The recent revival of this strategy (7), brought about by advances in the understanding of antigen presentation and lymphocyte-mediated cell killing, holds the promise of a rational and curative therapy. Since this goal remains to be reached, it is still far from certain that intelligent manipulation of the immune system will prove sufficient for a complete and assured cure of most cancers.

Might there be a generally applicable strategy for eliminating (or modifying) dangerous cells that is distinct from the existing approaches? Described below are the concepts of such a strategy.

**Indelins: Comtoxins That Bear Degradation Signals.** The main idea of this work is that the property of codominance, possessed by many signals in proteins and other biopolymers, can be utilized to produce therapeutic agents of previously unattainable selectivity. Figs. 1–4 illustrate the designs of these reagents, termed comtoxins (*codominance-mediated toxins*). We begin by considering comtoxins that utilize degradation signals (degrons). These comtoxins are termed indelins (*intracellular degron-dependent, ligand-regulated toxins*). The crucial property of an indelin is that its intrinsic activity—e.g., toxicity—is the same in all cells, whereas its half-life (and, consequently, its steady-state level and overall toxicity) in a cell depends on the cell's protein composition, specifically on the presence of two or more proteins that have been chosen to define the profile of a cell to be eliminated (Fig. 1). The metabolic instability of indelins and the possibility of control-

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Abbreviation: NLS, nuclear localization signal.

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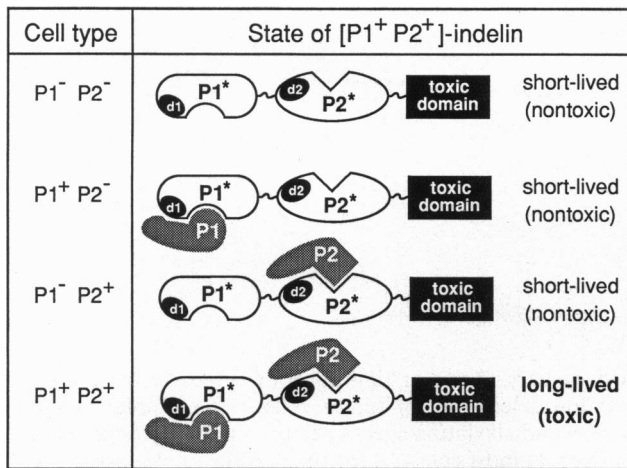


FIG. 1. An indelin (*intracellular degron-dependent, ligand-regulated toxin*) designed to kill P1<sup>+</sup>P2<sup>+</sup> cells (cells that express proteins P1 and P2) but to spare the other cell types (P1<sup>+</sup>P2<sup>-</sup>, P1<sup>-</sup>P2<sup>+</sup>, and P1<sup>-</sup>P2<sup>-</sup>) contains a cytotoxic effector domain (see text) and two degradation signals (degrons), d1 and d2, placed within or near two domains P1\* and P2\* that bind, respectively, to P1 and P2. In cells other than P1<sup>+</sup>P2<sup>+</sup>, at least one of the indelin's degrons, d1 and/or d2, is active (unobstructed), resulting in a short-lived and therefore relatively nontoxic indelin. By contrast, in P1<sup>+</sup>P2<sup>+</sup> cells both d1 and d2 would be masked by P1 and P2 (bound, respectively, to the indelin's P1\* and P2\* domains), resulting in a long-lived and therefore toxic indelin. This design requires that P1, P2, and the indelin reside in the same compartment, the one in which the indelin's toxic domain exerts its effect. In addition, the degrons d1 and d2 must be active in the relevant compartment; they may or may not be identical—the design is compatible with either choice.

ling this instability result from the presence of appropriately positioned degrons. A degron is an amino acid sequence, a conformational determinant, or a chemically modified protein structure that renders a protein short-lived (8). The concept of indelins stems from the following property of degradation signals: each degron in a short-lived protein bearing two or more degrons can function independently of other degrons in the same protein; i.e., multiple degrons of a protein can be codominant.

An indelin designed to kill cells that express proteins P1 and P2 but to spare cells that lack P1 and/or P2 (Fig. 1) is a multidomain fusion containing a cytotoxic effector domain (for example, a ricin or a *Pseudomonas* toxin; see below) and two degrons placed within or near two domains P1\* and P2\* that bind, respectively, to P1 and P2. An indelin is delivered into cells via routes employed with immunotoxins (5, 6) or through the use of expression vectors (more about this below). In a cell containing both P1 and P2, these proteins would bind to their respective ligands P1\* and P2\* in the indelin molecule and sterically mask the nearby (appropriately positioned) degrons, resulting in a long-lived and therefore toxic indelin. By contrast, in a cell lacking P1 and/or P2, at least one of the indelin's degrons would be active (unobstructed), yielding a short-lived and therefore relatively nontoxic indelin (Fig. 1). For an indelin (or another comtoxin) to be effective, the differences among cells in their levels of P1 and P2 proteins need not be all-or-none. Specifically, these differences should be large enough to cause the concentration of indelin (within the temporal window of a treatment) to be lethal in a targeted cell population but lower than lethal in cells containing smaller amounts of P1 and/or P2. Since other biopolymers, for example, RNA, can also fold into ligand-binding domains and bear signals such as degrons, nucleic acid-based comtoxins should be feasible as well.

**Codominance of Degrons.** The best understood intracellular degradation signal, called the N-degron, comprises a destabi-

lizing N-terminal residue and an internal lysine (or lysines) of a protein substrate (8). A set of N-degrons bearing different destabilizing residues is referred to as the N-end rule—a relation between the metabolic stability of a protein and the identity of its N-terminal residue (8). The *in vivo* half-life of a protein bearing a strongly destabilizing N-terminal residue such as arginine can be as short as 1 min, whereas an otherwise identical protein bearing a stabilizing N-terminal residue such as valine has a half-life of >20 hr, resulting in a >1000-fold difference between the steady-state concentrations of these proteins (8). The lysine residue of an N-degron is the site of formation of a multiubiquitin chain, which is required for the substrate's degradation by the 26S proteasome—a multicatalytic, multisubunit protease (9). Ubiquitin is a protein whose covalent conjugation to other proteins plays a role in a number of processes, primarily through routes that involve protein degradation (8, 9). At least one of the naturally short-lived proteins, the yeast Mata2 transcriptional repressor, bears two degradation signals located in two different regions of Mata2 and targeted by two distinct ubiquitin-dependent pathways (10).

This mechanistic understanding, while still rudimentary, is sufficient for concluding that spatially distinct degrons in the same intracellular protein can be codominant—i.e., that they can target the protein for degradation independently of each other. The property of codominance underlies the concept of indelins (Fig. 1). Another feature of ubiquitin-dependent pathways that is also central to the concept of indelins is processivity, the ability of these pathways to initiate and complete the degradation of a protein to short peptides after recognizing a protein's degron (8, 9).

**Intralins: Comtoxins That Bear Translocation Signals.** The property of codominance is not confined to degradation signals. For reasons analogous to those considered above for degrons, the signals that confer on a protein the ability to enter membrane-enclosed compartments (11, 12) should be able to function independently of each other if they are present in spatially distinct regions of the same protein. Comtoxins that utilize translocation signals are termed intralins (*intracellular translocation signal-dependent, ligand-regulated toxins*). The discussion below is confined to intralins that bear nuclear localization signals (NLSs).

Proteins smaller than ≈60 kDa can enter the nucleus by diffusing through the nuclear pores, but the pore-mediated transport of a larger protein requires the presence of at least one NLS accessible to components of the nuclear translocation system (12). NLSs are short sequences (10–20 residues) rich in lysine and arginine; their steric accessibility in a target protein appears to be sufficient for their activity as nuclear translocation signals (12). Many NLS-bearing proteins enter the nucleus shortly after their synthesis in the cytosol, but the transport of some proteins is not constitutive: their NLSs have to be “activated,” often by unmasking sterically shielded NLSs (12–14).

An example of a NLS-bearing intralin is a fusion containing an effector domain that is toxic in the cytosol but not in the nucleus (see below), and two NLSs placed within or near two domains P1\* and P2\* that bind, respectively, to P1 and P2—cytosolic proteins that define the target specificity of the fusion (Fig. 2A). If the cell's cytosol contains both P1 and P2, these proteins would bind to their respective ligands P1\* and P2\* in the intralin molecule and sterically mask the nearby (appropriately positioned) NLSs, resulting in a cytosolic and therefore toxic intralin (Fig. 2A). By contrast, if the cell's cytosol lacks P1 and/or P2, at least one of the intralin's NLSs would be active (unobstructed), resulting in a nuclear and therefore relatively nontoxic intralin.

Toxic proteins whose substrates are located in the cytosol but not in the nucleus include the diphtheria toxin and the *Pseudomonas* exotoxin A, both of which inhibit protein syn-

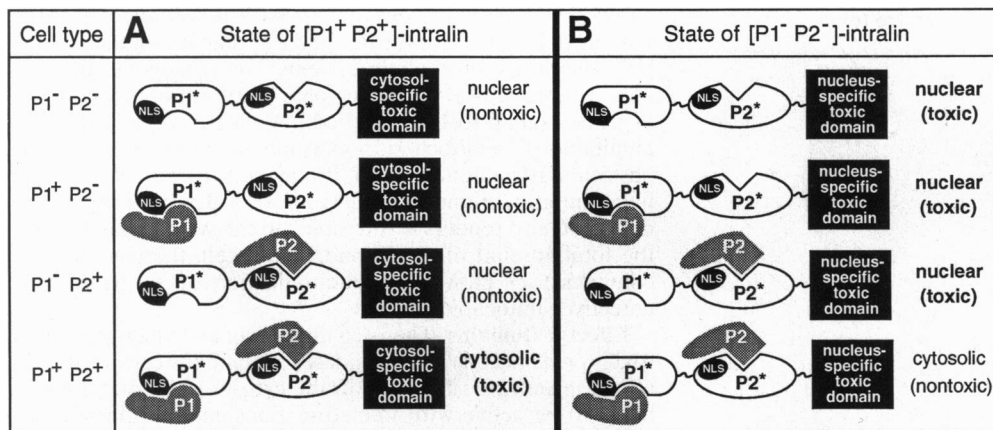


FIG. 2. (A) An intralin (intracellular translocation signal-dependent, ligand-regulated toxin) designed to kill exclusively P1<sup>+</sup>P2<sup>+</sup> cells contains a cytosol-specific effector domain and two NLSs, placed within or near two domains P1\* and P2\*, that bind, respectively, to proteins P1 and P2, which must be located at least in the cytosol. (B) An intralin whose toxic domain is nucleus-specific would kill P1<sup>+</sup>P2<sup>-</sup>, P1<sup>-</sup>P2<sup>+</sup>, and P1<sup>-</sup>P2<sup>-</sup> cells but would spare P1<sup>+</sup>P2<sup>+</sup> cells.

thesis by ADP-ribosylating (and thereby inactivating) elongation factor 2 (5, 6). Since the bulk of elongation factor 2 is cytosolic, the translocation of intralin containing a *Pseudomonas*-type toxic domain from the cytosol to the nucleus would physically separate a toxin from its substrate.

**Inverting the Selectivity of an Intralin.** An intralin analogous to the one above but bearing an effector domain that is toxic in the nucleus but not in the cytosol (see below) would kill cells that lack P1 and/or P2—the selectivity opposite to that of the intralin considered above (Fig. 2A vs. Fig. 2B). The ability to target cells that lack a set of predetermined proteins is important because cancer cells lack (or contain functionally inactive versions of) certain regulatory proteins (“tumor suppressors”) that are present in the normal progenitors of these cells (1–3).

**Indelins and Intralins Bearing a Single P\*-Type Domain.** Counterparts of constructs in Figs. 1 and 2 that bear a single P\*-type domain can function as indelins or intralins; however, these new drugs are not yet comtoxins (the mechanics of codominance requires more than one P\*-type domain). At the same time, the ligand-controlled toxicity of these drugs would be analogous to that of comtoxins. Thus, in addition to being an intermediate in the construction of a comtoxin, an indelin or intralin bearing a single P\*-type domain can be used as a drug specific for a single intracellular target.

**Combinatorial Selectivity of Hybrid Comtoxins.** Consider a comtoxin that contains an effector domain which is toxic in the nucleus but not in the cytosol, a degron placed within or near domain P1\*, and a NLS placed within or near domain P2\* (Fig. 3). As before, the P1\* and P2\* domains should be able to bind, to intracellular proteins P1 and P2, respectively, in contrast to a “pure” indelin or intralin (Figs. 1 and 2), this “hybrid” comtoxin would kill exclusively cells that contain nuclear protein P1 but lack cytosolic protein P2, for only in such cells would the comtoxin be both nuclear (because its NLS is not masked, owing to the absence of P2) and long-lived (because its degron is masked by the P1–P1\* complex) (Fig. 3). (The constraints on localization of P1 and P2 are actually less stringent than stated here; see Fig. 3 legend.)

Nucleus-specific effectors include deoxyribonucleases, either relatively nonspecific ones or restriction endonucleases. Indeed, *EcoRI* has been shown to cleave nuclear DNA *in vivo* (in the yeast *Saccharomyces cerevisiae*), killing the cells (15).

**Split Comtoxins.** Fig. 4 illustrates the idea of a ligand-sensitive split toxin. A single-domain protein whose subdomains are separated by a conformationally flexible insert can adopt a (nearly) normal conformation, in which the insert is extruded to the outside of the folded domain. For example, the *in vivo* folding of ubiquitin, a 76-residue protein, was shown to be virtually unperturbed by the insertion of an unrelated 80-residue sequence at a site between the two subdomains of

ubiquitin (16). The idea of a split comtoxin (Fig. 4) stems from these and analogous data (cited in ref. 16) and also from the notion that the extent of conformational flexibility of an insert between two subdomains of a protein should influence the protein’s folding. In particular, a conformationally rigid insert would be expected to perturb or preclude the coalescence of the protein’s subdomains.

In Fig. 4, two subdomains of a toxic domain are separated by a sequence that contains a binding site (P1\*) for an intracellular protein P1. Unlike the P1\* domains of other comtoxins (Figs. 1–3), the P1\* site of a split toxin (Fig. 4) should be a relatively short (peptide-size) region that remains conformationally flexible unless it is bound by P1. The construct of Fig. 4 would be toxic in cells that lack P1 but relatively nontoxic in P1-containing cells. For a split toxin to work, the affinity between subdomains of a toxic domain should be low enough to make their interaction substantially reversible, precluding irreversible activation of the toxic domain before its encounter with P1 (Fig. 4). This affinity can be adjusted, if necessary,

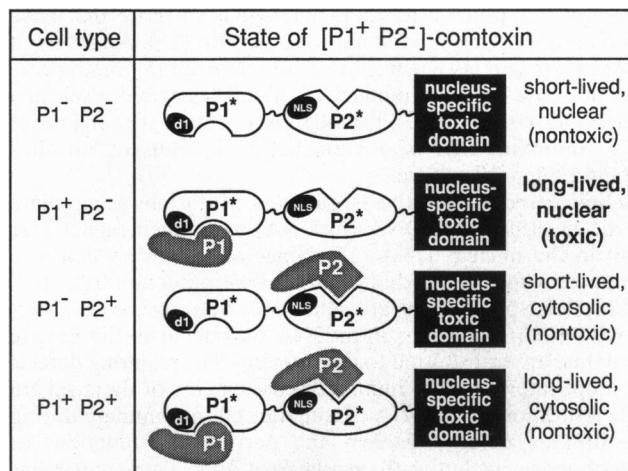


FIG. 3. A hybrid comtoxin that bears signals utilized in both indelins and intralins (Figs. 1 and 2) contains an effector domain that is active in the nucleus but not in the cytosol (see text), a degron placed within or near domain P1\*, and a NLS placed within or near the domain P2\*. P1\* and P2\* bind, respectively, to intracellular proteins P1 and P2. As described in the diagram, this comtoxin would kill exclusively P1<sup>+</sup>P2<sup>-</sup> cells. In these cells, P1 must be located at least in the nucleus, whereas P2 must be a cytosolic protein. Another constraint is that the degron d1 must be active at least in the nucleus. The actually indicated state of comtoxin in P1<sup>+</sup>P2<sup>+</sup> cells requires that P1 is present in both the cytosol and the nucleus and that the degron d1 is active in both of these compartments. If P1 is present largely in the nucleus, or if d1 is active only in the nucleus, the metabolic properties of this comtoxin would differ from those indicated, but its selectivity (killing exclusively P1<sup>+</sup>P2<sup>-</sup> cells) would remain the same.

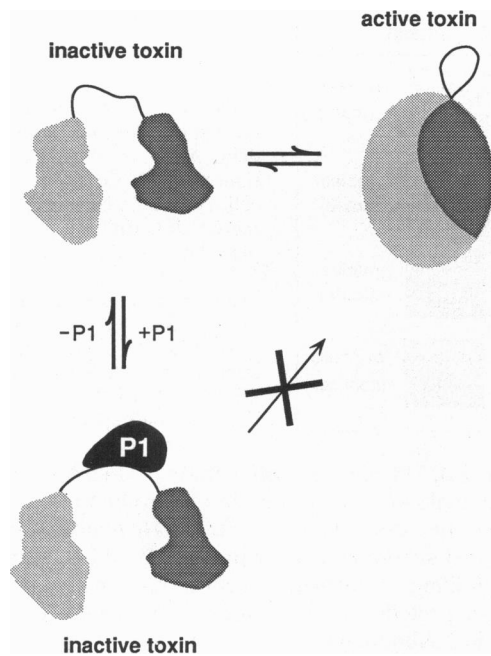


FIG. 4. Split toxin. In this design, two subdomains of a toxic effector domain are separated by an insert whose sequence contains a binding site for an intracellular protein P1. Unlike the P1\* domains of comtoxins in Figs. 1–3, the P1-binding site of a split toxin is a relatively short (peptide-size) region that remains conformationally flexible unless it is bound by P1. For this design to work, the affinity between subdomains of a toxic domain should be low enough to make their interaction substantially reversible (see text). A flexible insert between subdomains of a toxin can also contain two binding sites for intracellular proteins P1 and P2, arranged so that the binding of either P1 or P2 would be sufficient to impair the reconstitution of active toxin. The resulting comtoxin would kill exclusively cells that lack both P1 and P2.

through mutational alterations analogous to those that have been used with the subdomains of ubiquitin (16). An insert of a split toxin can also bear more than one protein-binding site, in which case the codominance of these sites would result in a split comtoxin (see Fig. 4 legend). More elaborate versions of split comtoxins that bear elements of indelins or intralins should be feasible as well.

**Elimination of Defective Organelles.** Mutations accumulate in the mitochondrial DNA (mtDNA) at a much higher rate than in the nuclear DNA (17). Since almost every cell in a metazoan organism harbors hundreds of mitochondria, their intracellular populations are genetically heterogeneous (“heteroplasmic”). Mutations in mtDNA that occur in the female germ line are transmitted to the progeny. The resulting defects are implicated in >100 human diseases, many of them severe (17). Mutations in mtDNA of somatic cells accumulate during the lifetime of an organism and perturb the functions of mitochondria, including the synthesis of ATP. These mutations are among the causes of time-dependent decline in the performance of organs that is the hallmark of aging (18).

Disruptive effects of malfunctioning organelles are beyond the reach of current therapies. Remarkably, this problem might become addressable with comtoxins that utilize the mitochondrial translocation pathway (11) to enter mitochondria and selectively destroy those among them that no longer produce a “required” amount of ATP. The likely common features of defective mitochondria include elevated levels of mitochondrial stress proteins. A mitochondrial comtoxin can be, for example, an indelin containing a mitochondrial presequence (11), a toxic effector domain, domains P1\* and P2\* that bind to mitochondrial stress proteins P1 and P2 (whose levels are low in the normal mitochondria but high in defective ones),

and mitochondrial degrens that are sterically masked upon the binding of P1 and P2 to the P1\* and P2\* domains of the indelin. [Mitochondrial proteolytic systems are analogous, but not identical, to those of the cytosol (19); intramitochondrial degrens remain to be identified.] A therapeutically relevant elimination of a mitochondrion would not necessarily entail its physical destruction. Instead, it may be sufficient if the toxin halts the growth and multiplication of a defective mitochondrion and also renders it “invisible” to the system that controls the total amount of mitochondria in a cell, thereby allowing compensatory growth and multiplication by surviving (less defective) mitochondria.

**Effector Domains.** The A chain of ricin and analogous plant toxins, which act as N-glycosidases specific for certain sites in rRNA, inactivate ribosomes in the cytosol (5, 6) and are also likely to be active with immature ribosomes of the nucleus. Other examples of toxins whose substrates are present in both the cytosol and the nucleus include ribonucleases such as RNase A and barnase, which have been used to produce chimeric toxins (6).

Designs of intralins (Fig. 2), hybrid comtoxins (Fig. 3), and split comtoxins (Fig. 4) require effectors that are toxic exclusively in the cytosol or exclusively in the nucleus. Examples of such effectors were discussed above. Since the goal of therapy is to render dangerous cells harmless, the set of useful effector domains is not confined to cytotoxic proteins; it may include, for instance, transcription factors whose presence in a cell results in growth arrest and terminal differentiation.

**Designing P\*-Type Domains.** These domains of a comtoxin (Figs. 1–3) bind to specific intracellular proteins (P1, P2, etc.). A homodimerization of P\*-type domains is undesirable because it would interfere with the function of a comtoxin. Thus, a P\*-type domain should be able to form a heterodimer with its intracellular partner (a P1-type protein) while not forming a high-affinity homodimer. Possible designs of a P\*-type domain include the following.

- (i) A natural protein ligand of a P1-type protein.
- (ii) A peptide-size fragment of the natural ligand that retains affinity for a P1-type protein (Fig. 4).
- (iii) A single-chain antibody (5, 6) specific for a P1-type protein. This class of P\*-type domains includes single-chain antibodies to the junctional regions of tumor-specific fusion proteins produced by tumorigenic chromosome translocations (1–3). These mutant proteins form early in the evolution of a tumor cell lineage, are likely to be required for the malignant phenotype (2), and therefore are an especially pertinent class of P1-type proteins.
- (iv) A nonpeptide, low molecular weight ligand of a P1-type protein. This possibility is confined to directly delivered (as distinguished from expression-based) comtoxins.

**Target Cells and Intracellular Ligands.** The detailed protein composition of specific cell types, in particular of normal and tumor-derived human cells, is being determined by a number of laboratories, but the information gathered thus far is still sketchy for most cell types. Therefore a useful short-term strategy would be to choose the intracellular ligands of a comtoxin from a set of intracellular proteins that are already known to be either overproduced in the target cells or absent from them; the latter proteins should be present in most (not necessarily all) nontarget cells.

Consider a tumor cell lacking the wild-type version of a tumor-suppressor protein (expressed in the normal progenitors of this cell) and overproducing another protein, perhaps as a result of a gene amplification that contributed to the cell’s malignant phenotype. For example, many (but not all) human breast carcinomas lack the tumor-suppressor protein p53 or contain its functionally inactive variants (3). Further, many of these tumors overproduce the protein c-Myc (in addition to several other proteins) (3). A comtoxin that kills cells which contain a P1 protein but lack a P2 protein has the requisite

selectivity for such a setting. The comtoxin's P1\* domain would bind to c-Myc, while the comtoxin's P2\* domain would bind to wild-type p53 but not to its mutant variant in a given carcinoma. Even nonselective intracellular delivery of this comtoxin would kill Myc-overexpressing, p53-lacking carcinoma cells but spare most, if not all, other cells of the organism, because most normal cells contain p53; the few, if any, normal cell types that lack p53 are unlikely to overproduce c-Myc (1–3). Moreover, the selectivity of this comtoxin can be increased further, if necessary, by adding to it a degran- or NLS-containing P3\* domain that binds to a third intracellular protein, P3, chosen to sharpen the description of target cells.

This example illustrates strategies that can be used to select an optimal set of intracellular ligands for comtoxins against specific cancers, against cells infected by a specific virus, or against other undesirable cells. Examples of the latter are cells that form atherosclerotic plaques, and cells of neovascular endothelium, whose selective ablation would cut off the blood supply to a metastatic tumor (20).

**Delivery to Targets.** For delivery via the intravascular route (4), a comtoxin should possess not only a toxic domain but also a domain that mediates the translocation of a fusion from the cell surface to the cytosol. This aspect of comtoxins is confined to direct-delivery (as distinguished from expression-based) strategies and is similar to the analogous aspects of current immunotoxins (5, 6). Comtoxins can also be delivered into cells through the intermediacy of expression vectors. Both of these approaches are a part of ongoing efforts to improve bioavailability of protein drugs used in medical interventions, from cytotoxic treatments to gene therapies (5, 6, 21, 22). The problem of insufficient selectivity is common to all of the current cytotoxic strategies: once the effector reaches its intended intracellular compartment, the cell is likely to be killed irrespective of whether it was a target or an innocent bystander. For example, one drawback of the current immunotoxins is their nonspecific toxicity—largely, but not only, to the liver (5, 6). This toxicity, which imposes a limit on both duration and intensity of treatments, stems in part from the clearance of an intravenously administered immunotoxin by cells of the reticuloendothelial system (5). By contrast, even nonselective delivery of a comtoxin (Figs. 1–3) would not affect most nontarget cells.

Comtoxins designed for delivery by an expression vector would lack the “compartment-crossing” domain required for their directly delivered counterparts. Recent advances in the design of viral and plasmid-based vectors are bringing closer the possibility of tailor-made, nonreplicating vectors that can transfect both growing and quiescent cells, elicit little or no immune response, and are either specific for cells that bear a predetermined surface marker or almost nonselective. Such vectors, under development for gene therapy and other applications (21, 22), should also yield powerful methods for the delivery of comtoxins.

**Concluding Remarks.** The main idea of this work is that the property of codominance, characteristic of signals such as degraons and NLSs, can be utilized to produce a new class of therapeutic agents—indelins, intralins, and other comtoxins (Figs. 1–4). A feature of these reagents is their potentially unlimited, combinatorial selectivity for targets that can be

either whole cells or specific organelles. The sources of this selectivity are distinct from mechanisms that underlie immunological recognition, suggesting that comtoxins can be designed to do things that the immune system cannot. For example, even if the immune system will someday be harnessed to eliminate any set of undesirable cells in an organism without injuring other cells—a most optimistic assumption—there would still be the problem of “rejuvenating” otherwise normal cells by selectively destroying their defective organelles. As discussed above, a cell-level approach would be inadequate here, because every cell in an aging organism contains both healthy and defective mitochondria. This and analogous problems are not considered in the context of therapy today but may become addressable through the development of comtoxin technology. Meanwhile, the nearest testing grounds for this approach will be cell-specific therapies, in which comtoxins are directed against virus-infected and cancer cells.

The features that set comtoxins apart from earlier therapeutic agents are the potentially unlimited selectivity of a comtoxin and the possibility of incremental increases in selectivity through the use of the same strategy that yielded a preceding, less selective drug. If the properties of comtoxins live up to the premise of their design, this approach may help eliminate the problem of side effects—the bane of today's therapies—for even nonselective delivery of a comtoxin would not affect cells whose protein “signatures” differ from the targeted one.

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