

The zinc finger nuclease monopoly

A decade of sound science and aggressive deal making has given Sangamo Biosciences a stranglehold on zinc finger technologies. Now, academic labs that helped build Sangamo's empire want in on the action. Are the ingredients ripe for a revolt that could break the company's monopoly? Christopher Thomas Scott investigates.

In Richmond, California, just across the bay from San Francisco, the secrets of a cutting-edge technology that could transform gene therapy lie hidden in the intellectual property (IP) vaults of a small biotech company. Sangamo Biosciences holds key patents and trade secrets not only on the design of zinc fingers and zinc finger chimeric endonucleases (ZFNs), but also on their uses in drug discovery and the regulation of gene expression (Table 1). The technology has prompted excitement in gene therapy circles, not least because of the recent demonstration in a *Nature* paper of highly efficient and permanent correction of a mutated gene associated with severe combined immune deficiency (SCID)¹. Will Sangamo hold the keys to the zinc finger kingdom, or will it yield to pressure to make its technologies widely available?

Unbounded excitement, bounded technology

Engineering a zinc finger nuclease (ZFN) to introduce a double-strand break at a specific single chromosomal locus and induce homology-directed repair with an exogenously added donor DNA sequence promises a completely new means of gene replacement in cells and whole organisms. Unlike traditional gene therapy, ZFN therapy may avoid problems of insertional mutagenesis that have plagued certain retroviral gene therapy trials in SCID², eliminate the need for large and unwieldy exogenous donor DNA sequences (which encode both protein and appropriate regulatory molecules) and not suffer from inappropriate tissue specificity, timing and level and duration of expression.

In April, Sangamo demonstrated for the first time that ZFNs could correct the gene encoding human interleukin 2 receptor- γ (IL2R γ), which underlies X-linked SCID³. But the company is already busy thinking beyond remedies for SCID. One potential application of the ZFN technology, for example,

would be to confer long-term resistance to HIV by disabling the gene for CC-chemokine receptor 5 (CCR5) by nonhomologous end joining in stem cells (Box 1).

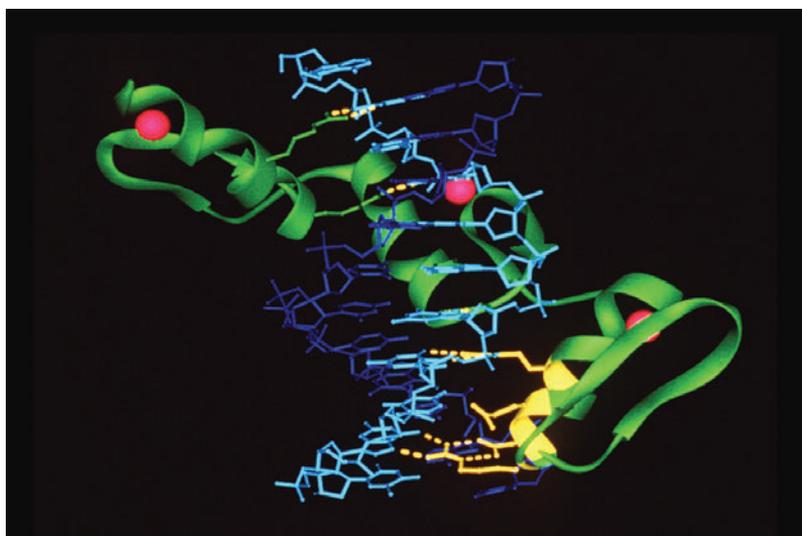
Meanwhile, Sangamo's proprietary database of zinc fingers has academic experts both excited and nervous. Researchers want access to the library to optimize and refine the ZFN platform for applications in gene therapy, gene regulation and gene replacement. Some are already tied to Sangamo by licensing agreements (through their respective institutions). Yet even those whose inventions are part of the patent estate instrumental to the company's fortunes are often not privy to key aspects of the technology; many, for example, lack the know-how that made the IL-2R γ gene replacement experiments in SCID-derived cells possible³. Sangamo says it must control its IP to maintain value and ensure its survival; making the IP freely available to academic laboratories relinquishes that control. It is a familiar refrain at the interface of industry and academia.

A delicate balance

At issue is Sangamo's rule-based library of combinations of two and three zinc fingers for targeting longer DNA sequences. Thirty-two GNN and ANN triplets—the basic unit of recognition of zinc fingers (see p. 967)—are in the public domain; a complete set of 64 with operating instructions would, in principle, be enough to design ZFNs with uncommon sequence specificity, especially to those disease genes buried deep within the human genome's three billion base pairs. The zinc finger grapevine is buzzing with rumors that Sangamo is slow to respond to requests for materials and is making negotiations difficult with licensees.

A failed deal with Phytodyne, a plant biotech company based in Ames, Iowa, has added to the unsettled mood. Its founder, Dan Voytas, a respected gene-targeting expert, has left the company to return to the University of Iowa. Voytas had negotiated an exclusive license to an invention made by the University of Utah's Dana Carroll, who demonstrated targeted gene replacement in whole organisms—but he also needed a sublicense to Sangamo's ZFN patent based on work by Baltimore's Johns Hopkins University's Srinivasan (Chandra) Chandrasegaran, a ZFN pioneer and former advisor to Sangamo. The deal fell through. Reportedly short on cash, Phytodyne is not expected to survive. "The Sangamo patent was essential, and price was a big factor," Voytas says. Sangamo CEO Edward Lanphier agrees that money was the key. "We felt Phytodyne is very strong scientifically, but doesn't have financial staying power."

To its credit, Sangamo has put dozens of materials transfer agreements in place with



Zinc fingers doing their magic on DNA.

Sangamo Biosciences, Inc.

Table 1 Selected recent patents issued to Sangamo

Title	Patent number	Year of issue	Year of expiration
Regulation of endogenous gene expression in cells using zinc finger proteins	6,824,978	2004	2019
Iterative optimization in the design of binding proteins	6,794,136	2004	2020
Selection of sites for targeting by zinc finger proteins and methods of designing zinc finger proteins to bind to preselected sites	6,785,613	2004	2019
Functional genomics using zinc finger proteins	6,777,185	2004	2019
Nucleic acid binding proteins (zinc finger proteins design rules)	6,746,838	2004	2018
Screening system for zinc finger polypeptides for a desired binding ability	6,733,970	2004	2019
Regulation of endogenous gene expression in cells using zinc finger proteins	6,607,882	2003	2019
Functional genomics using zinc finger proteins	6,599,692	2003	2019
Methods of using randomized libraries of zinc finger proteins for the identification of gene function	6,503,717	2003	2020

universities. The dissatisfaction isn't with the agreements themselves but with the way that Sangamo controls the interaction. Investigators who want zinc fingers send the desired sequence to the company. The company builds the nucleases, and sends them back. The 'rule set' remains protected. "This ZFN stuff is really hard," says Sangamo's senior research director Phillip Gregory. "It is not trivial to make a nuclease that will do exactly what you want." The work is so exacting that the company has a core group of biochemists that do nothing but design, select and optimize chimeric nucleases. Gregory hopes that in the future, the library will be made public. "From a scientific point of view it would be great to have the archive public. The concern from the business side is that this is hard to do well, and we want people to be successful."

The natives grow restless

In the meantime, those eager to work with Sangamo wait. In California, Stanford University's Mark Kay is one of those stand-

ing in line. "They have good scientists doing strong science," the gene therapy expert says. "But they must get through this honeymoon period of excitement." The company will need to enlist collaborators like Kay as they move deeper into gene therapy territory. Despite the encouraging SCID results, the standard hurdles, such as targeting, delivery and immunogenicity, still apply³. Taken together, the message from the zinc finger community is that the number and productivity of Sangamo's current alliances may predict whether Sangamo will have academic partners to tackle thorny problems later.

The charged atmosphere has even provoked sleuthing among the academic labs. Chandrasegaran muses, "I hear Scripps Research Institute's Carlos Barbas has developed the other 32 triplets." Barbas' laboratory, which has the ability to design and assemble three-finger sets, is watched with great interest. "I've checked the patent records, and it looks like Carlos has a patent pending on CNN triplets. There are rumors he has the TNN triplets," says University of

Texas Southwestern's Matthew Porteus, co-investigator on April's *Nature* SCID paper³. Whether Barbas (who was unavailable for comment) has the remaining triplets is a mystery. Whether he will file them as inventions or release them to the public is a bigger question still.

Finally, there is the 'rule set.' Having access to the triplets is only half the battle—the real value is the informatics used for selection and assembly. Dana Carroll suggests Sangamo consider open access. "We'd be more comfortable if they were generous about letting others pursue the technology," he says. "We'd make faster progress if many people work on it." Chandrasegaran, although acknowledging the need to control IP, says, "They should open [the library] up and let everybody use it—or at least make it easier for people to use it."

The art of licensing and acquisitions

"Things are a bit lonely," frets Sangamo's Gregory. It is no wonder he feels left out. A decade of aggressive licensing and one very well-timed acquisition means his company is

Box 1 Diversification and development

The ZFN-induced repair of X-linked SCID overshadows the fact that 50% of Sangamo's scientists have been busy taking transcription factors to the clinic. In 2000, Edwards Life Sciences (a spinout of Baxter) inked the first therapeutics deal using a zinc finger protein transcription activator for vascular endothelial growth factor (VEGF) to promote angiogenesis. The \$25 million partnership propelled two phase 1 clinical trials for peripheral arterial disease, one at Duke University, the other at the US National Institutes of Health. Sangamo has opened its own VEGF trial at sites in Texas and San Diego. Gregory notes that the company's clinical research has revived the interest of pharmaceutical companies looking for collaboration. Indeed, Sangamo announced deals with Pfizer and Thousand Oaks, California-based Amgen this year. Lanphier is so confident of the partnering ability of his company that he has told Wall Street—

five analysts follow Sangamo's fortunes—to expect at least one more alliance in 2005 and others down the road.

On the balance sheet, the company looks more like a standard biotech gearing up for another round of financing. With \$20 million in cash and a two-year operating window, what will Lanphier do as Sangamo enters the next phase of development, including the treacherous waters of gene therapy? "This is the phase where I've had the most experience," he says. "If we're successful in the clinic, then our IP becomes a significant part of our asset value. The combination will give us cash and financing from the public markets." It seems so. With 30 issued patents, 70 patents pending, three open investigational new drug applications and preclinical results demonstrating the power of ZFN-mediated gene correction, Sangamo has the equivalent of three hotels on a purple swath of Park Place.

the only commercial player in a red-hot field of chimeric nucleases.

Gregory knows there are two edges to this sword. "We're fortunate," he says. "Our intellectual property is very robust." Robust indeed—the research director can't name a company he'd consider a competitor. The downside is that Sangamo's monopoly doesn't lend itself to unfettered collaboration. "It'd be nice to have plenty of people working on zinc fingers," he says.

How did Sangamo reach its catbird's seat? Credit CEO Edward Lanphier. In 1995, Lanphier was head of business development and chief financial officer of Alameda, California's Somatix Therapy, a vector-based gene therapy company. Responsible for the company's 'patent estate,' he licensed in technologies to compliment Somatix's foundational IP. Biotechs rarely survive without a strong IP portfolio: it is a crucial measure of value that can bring new capital and courtship opportunities with big pharma.

Somatix's proprietary core was a gene vector delivery system. Executing deals is what business development professionals are paid to do, and Lanphier found that he couldn't do the deals he wanted or had to strike multiple agreements that stacked royalty payments. "We had a great system, but limited access to proprietary genes," he recalls.

While beating the bushes, Lanphier noticed the work of Chandrasegaran, who had invented a method to fuse a DNA binding domain of 3 zinc fingers to a cleavage domain of bacterial type IIS restriction enzyme, *FokI*. Somatix wasn't interested in an enzyme that could bind DNA with amazing precision, but Lanphier was. He left Somatix to found Sangamo in 1995 with \$750,000 and an exclusive license to the Chandrasegaran patent (Somatix merged with Cell Genesys in 1997). From 1995–2000, the Johns Hopkins chemist sat on Sangamo's advisory board. He remembers the early touch-and-go period of the company fondly. "We had a lot of fun, keeping the company going, worrying about its survival."

Priming the pump

The association with Chandrasegaran was providential. Chandra, as peers and students call him, introduced Lanphier to the best zinc finger chemists in the world. Licensing agreements and critical hires soon followed. He met Cambridge-based Massachusetts Institute of Technology's (MIT's) Carl Pabo and Carlos Barbas, a young molecular biologist working at Scripps in La Jolla, California. Pabo, a world-renowned crystallographer and Howard Hughes investigator,

performed elegant work engineering zinc fingers to regulate gene expression. Later he joined Sangamo as chief science officer, and he now chairs the company's scientific advisory board. For his part, Barbas uses a phage display system to select single zinc fingers with high affinity and couples activator and repressor domains to the proteins to regulate gene expression.

Lanphier's energetic hoovering of transcription factors and ZFN technologies began to swell his patent coffers. Exclusive licensing deals with Matthew Porteus in 2003 and Dana Carroll in 2004 sewed up technologies that reduced ZFNs to practice. Porteus, then working in David Baltimore's laboratory at CalTech, was the first to show that ZFNs were active in human cells; Carroll perfected mutagenesis and targeted gene replacement in whole organisms, including *Drosophila melanogaster*.

A year after an initial public offering in 2000, Sangamo's cemented its dominance by acquiring London-based Gendaq, a highly respected gene regulation firm founded by

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Sir Aaron Klug, a 1982 Nobel Prize winner and former director of the Medical Research Council Laboratory of Molecular Biology in London. "At this stage, the platform needed work," Chandrasegaran recalls. "Once they got to Klug, they had the market cornered." The \$30 million buyout netted Sangamo a phage display methodology and rules for designing a modular library of two zinc finger sets, catalogued by amino acid substitutions in the protein's binding region. Company chemists added more sets and validated the library.

The result is the bedrock of Sangamo's IP: an informatics-powered commercial archive of two-finger zinc finger proteins. The system correlates specificity, affinity and binding characteristics for a chosen DNA target. Once selected, two or more zinc finger proteins can be joined to make four- or six-finger proteins. A pair of high specificity four-finger ZFNs (one manufactured for each half of the dimer) was used to correct the X-linked SCID gene, IL-2R γ , reported in the April issue of *Nature*³.

Shaking up empires

Can a patent empire be broken? The answer to this question depends on two things: demand and old-fashioned one-upsmanship. Those with sufficient expertise and resources who want something bad enough can attempt to design around, over, or above the state of the art. Dan Voytas says he will do just that. "I received a \$2 million National Science Foundation grant, and rather than pursue a commercial strategy, I've decided I'd rather disseminate the technology," he says. "We will design our own set of rules, software tools and vectors—anything that enables the technology—and make it available to as many people as possible."

Kathleen Williams, a Boston-based IP attorney and biochemist specializing in biotech patent estates, notes that Sangamo's IP position is unusually strong, especially when it comes to circumventing cDNA patents. No recombinant genes are necessary because zinc fingers do their work *in situ*. Zinc fingers free Lanphier from the yoke of in-licensing enabling technologies. "This is what our law promotes. It is a legal, smart and strategic way of making business goals effective" Williams says.

On the other hand, monopolistic practices invite competition, and in the case of academic laboratories, willful infringement. For decades, university laboratories have used patented methods and tools without a license. The private sector turns a blind eye, knowing that suing an ivory tower would be a public relations disaster. Despite this practice, Williams sees the zinc finger field as ripe for a fight. Claims to proteins and DNA are more narrowly written now than a decade ago, and as a result, easier to invent around. She contends there is plenty of federal circuit law that supports "design-arounds," adding, "Anything that can stand next to an existing invention as complimentary or alternative is strongly encouraged in the law."

The mere threat to innovate can spur a company holding a monopoly to loosen its grip. In the mid-1990s, Affymetrix's high prices and tough negotiating tactics prompted Stanford University's Patrick Brown to devise novel means for making gene chips, which he published on the Internet. That, along with a growing dissatisfaction from the genomics community, prompted the chip company to offer affordable licenses to nonprofit institutions. Similar pressures and an unsuccessful infringement suit against a score of academic laboratories forced Berkeley, California-based Cetus (which was bought out by Emeryville-based biotech Chiron), who then owned the patents, to distribute and discount its new PCR technology more broadly⁴.

Innovating around patents

Finally, consider the direct assault. The zinc finger rumblings have reached Canberra, Australia, where Cambia's Richard Jefferson, open-source guru⁵ is mulling over which biotech windmill is ripe for another tilt. Likewise, Jefferson's attempt to bypass Monsanto's *Agrobacterium tumefaciens* patent estate has caught the attention of stateside zinc finger protein laboratories⁶.

Jefferson argues that a monopoly isn't innate to the IP of a single company; the brute force of numbers and sheer innovation can break a monopoly. "There are more smart zinc finger and recombinant scientists in the world than Sangamo can ever hope to have inside their company," he argues. The first task, according to Jefferson, is to produce a detailed, publicly available "patent landscape" that describes the field. The second is to exploit the weaknesses through a collaborative open source project that invents beyond the monopoly. New technologies are then made freely available.

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Jefferson and patent reformer David Martin, of the University of Virginia's business school, believe that patents are overvalued by investors who don't appreciate that under a challenge, inventions can be found to be worth little or nothing at all. And, the courts invalidate or alter around half of litigated patents. Martin noted in a June *Washington Monthly* interview that the Chinese are already probing the margins of American monopolies, including Pfizer's (New York) Viagra (sildenafil citrate) and Lipitor (atorvastatin)⁷.

Sangamo's Lanphier and Gregory maintain that their academic agreements are essential to the company's success. Now that ZFNs have cleared the first therapeutic proof-of-concept, physician-scientists such as Porteus have begun to shift their attention to the needs of patients—in his case, sick children with genetic diseases. "An open resource would generate enormous good will," Porteus says. In the end, it may be the scientists who are part of Sangamo's patent estate, rather than the patents themselves, who determine the direction of this exciting field.

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