machinery during heterochromatin formation [14]. In the neocentromere on Chromosome 10 the heterochromatin protein HP1 does not co-localize with the CENPA-containing nucleosomes. The predominant positions that affect variegation of transgene expression in mice are pericentric heterochromatin, but chromosome engineering experiments have failed to demonstrate silencing when these genes are placed close to human alphoid DNA.

Conclusions

What practical relevance does this have? Manipulated or artificial chromosomes have been produced in several laboratories with the aim, at least in part, of providing an expression platform for genes that avoids both integration into host-cell genomes and position effects. Paradoxically, a potential problem with this approach is that genes placed in close proximity to a centromere might be silenced by centromeric heterochromatin, but this now seems less of a concern. A better understanding of the requirements of a centromere in terms of formation, propagation and function in cell division is needed to produce better artificial chromosomes. The approaches discussed here that are used to define molecular markers of centromeric DNA, coupled with biochemistry and biophysics of centromeric chromatin, are a part of that process.

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Synthetic biology evolves

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Synthetic biology is advancing rapidly as biologists, physicists and engineers are combining their efforts to understand and program cell function. By characterizing isolated genetic components or modules, experimentalists have paved the way for more quantitative analyses of genetic networks. A recent paper presents a method of computational, or *in silico*, evolution in which a set of components can evolve into networks that display desired behaviors. An integrated approach that includes a strategy of *in silico* design by evolution, together with efforts exploiting directed evolution *in vivo*, is likely to be the next step in the evolution of synthetic biology.

It is clear that the most successful techniques for manipulating biological systems exploit what nature already does well. For example, by harnessing enzymes that selectively cut DNA molecules as part of the bacterial ‘immune system’, clever researchers helped to spark the revolutionary field of recombinant DNA technology. There are numerous other examples in which characterized components, which have been tailor-made for specialized function through Nature’s evolutionary process, have been used in novel ways for biotechnological and medical applications. This recipe for success is being employed in the emerging field of synthetic biology; researchers are reassembling well-characterized genetic components into artificial networks that perform prescribed functions *in vivo* [1,2]. Presently, these efforts are primarily driven by a rational approach to gene circuit design,

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where well-described parts are assembled based on predictions borne out of mathematical models of circuit behavior [3–10]. However, recent work by François and Hakim [11] offers a complementary *in silico* approach to network design that incorporates evolution in a new technique that mimics how cells themselves undergo mutational selection.

Engineering genetic networks *in vivo*

Decades of research, starting with the ground-breaking work of pioneers like François Jacob and Jacques Monod [12], have elucidated the basic components and logic of gene regulation. In conjunction with these efforts, and in part owing to the vast amount of information gleaned from ongoing work to decipher the functions of gene networks, researchers are developing increasingly sophisticated models of cellular networks. Together, these advances have helped to foster the field of synthetic biology, in which artificial circuits are constructed based on *in silico* descriptions of network function in an attempt to achieve a level of understanding that will enable the creation of fully ‘programmable’ cells. Achieving this lofty goal requires not only a detailed understanding of how simple components interact within a network, but also an understanding of how these networks interact within the complex cellular environment. Despite these challenges, recent studies have shown that predictable, albeit simple, cell behavior is readily achievable.

Several simple genetic networks (or modules) have been constructed *in vivo* using a rational approach to artificial gene-circuit design. Artificial networks, including feedback systems [6–8], toggle switches [4,9], oscillators [3,9] and cell–cell communication systems [5,10], were constructed using predictive models that uncovered network behavior and helped to guide experimental design. This approach performed remarkably well, as demonstrated by the success of circuit performance *in vivo*. However, a limiting factor is the inability to predict precisely how a circuit will function within the cellular environment because of the enormous complexity of living organisms. It seems that simple engineered networks require extensive tinkering with their elementary components to obtain the desired behavior in the face of unknown interactions and the experimentally observed noise in gene expression [13–15]. One way to simplify the task of artificial circuit construction involves a combinatorial approach: isolated components are assembled *in vitro*, and the resulting networks are inserted into a cell in which circuit behaviors can be identified [16]. This approach can circumvent the tedious task of constructing one particular circuit to perform a desired function by providing a multitude of circuits spanning the range of available components and behaviors. Although this method proved useful for the creation of simple logic-gate networks [16], the requirements of large-scale screening techniques can prove challenging when trying to create complex networks of more diverse functions.

A method of construction that does not necessitate a prior knowledge of the details of circuit function within the global cellular environment involves using directed evolution – a process that takes advantage of the ability of a cell to survive under selective pressure. Directed evolution is often associated with techniques used to improve protein function by DNA shuffling *in vitro* [17], as well as to generate new functional nucleic acid sequences such as aptamers and ribozymes [18]. Recently, an approach involving directed evolution has been applied to a rationally designed synthetic network *in vivo* [19]. In this case, the circuit was already well described and targeted mutation, together with screening based on fluorescent reporter properties, was used to make a non-functional circuit functional. This approach involved a combination of rational circuit design and evolution to improve performance. In this way, it might serve to augment previous approaches that combine predictive models with experiments to produce a network with a particular function.

In silico evolution of genetic networks

François and Hakim [11] describe a procedure aimed to reproduce *in silico* the feature that drives the design of genetic network architectures *in vivo*: evolution. They present a computational algorithm that creates small gene networks as depicted in Figure 1. Their algorithm begins with a collection of genes and proteins (accompanied by deterministic rate equations describing their interactions) that subsequently undergo alternate phases of mutational ‘growth’ and ‘selection’ to evolve into networks of a specified function. The evolutionary process is repeated until the desired networks are created. This approach

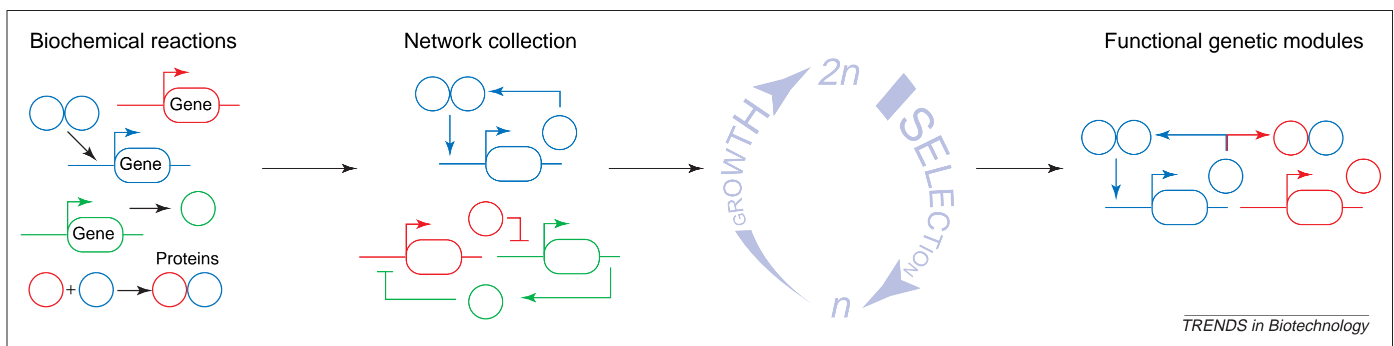

Figure 1. *In silico* evolution procedure of genetic networks as described by François and Hakim [11]. Genes and proteins are assembled and described according to their biochemical reactions, forming a collection of networks. These networks then serve as the input to the iterative ‘growth’ and ‘selection’ procedure, where ‘n’ networks are doubled through mutational growth and subsequently pruned (n = 100). After successive rounds of ‘growth’ and ‘selection’, functional genetic modules of prescribed behavior are created.

presents an interesting way to streamline artificial circuit design that could bridge the gaps in our understanding of native biological networks and expedite efforts to engineer cellular control.

Although theoretical models have been used alongside experimental work to clarify qualitative features of synthetic gene-networks [3,4,6,7], provide quantitative understanding of modular components [8,20] and identify basic network building blocks [21,22], the algorithm generated by François and Hakim [11] is a step closer to simulating the natural process of circuit design. Cells are subject to a continuum of change that includes environmental stresses and genetic mutations that affect their ability to grow and propagate. The algorithm by François and Hakim mimics how cell populations might respond to evolutionary pressures by propagating networks *in silico* based on selected behavior. In the algorithm, the growth and selection phases are analogous to those that a cell population might undergo; genetic networks double in size through *in silico* growth of a mutated copy of each network in the collection. These mutations might include modified protein degradation rates (or other kinetic constants), or the introduction of a new gene or protein. Next, each network is evaluated by integrating its set of coupled differential equations. A scoring function is then assigned that ranks each network by specified behavior (e.g. bistable switching or oscillations). Based on this ranking, a network is either pruned or selected to proceed to the next round of growth and selection. This process is repeated until the algorithm converges on the networks that exhibit the prescribed behavior.

In letting the networks evolve into a collection of optimal architectures, the algorithm takes advantage of every available component and interaction. One of the most interesting results from this approach is the crucial role of post-transcriptional control in network function. To date, this feature has been largely absent in synthetic biology efforts that have primarily focused on transcriptional regulation [1,2]. François and Hakim show that many of their evolved genetic modules rely on post-transcriptional interactions to achieve the desired bistable states and oscillatory behavior [11]. Importantly, their algorithm most often produced *in silico* motifs for bistable networks that incorporated post-transcriptional control. Several of these general motifs have been shown to exist in *Escherichia coli, Xenopus laevis* and *Drosophila melanogaster*, providing further validation of their algorithm. Interestingly, the authors also show that multiple network motifs render the same desired behavior (i.e. bistable states). It would be useful to investigate – both *in silico* and *in vivo* – the stability of various motif architectures with similar dynamics, given recent experimental insights into the important role of noise in biological networks [13–15].

Concluding remarks

One of the greatest challenges facing the field of synthetic biology is the ability to construct larger and more complex networks of diverse components. Experimental efforts involving the generation of libraries of small networks through combinatorial synthesis [16], as well as the creation of genetic devices with a range of behaviors by screening networks with evolved genes [19], have made significant advances in artificial gene-network construction and function. However, the article by François and Hakim sets the stage for what could be the next experimental challenge: the development of streamlined techniques that use the cell to assemble, grow, evolve and select for genetic modules with desired behaviors. From a biotechnological perspective, this *in silico* algorithm – which can be easily scaled to include additional components and refined to account for more detailed cellular interactions – might inspire the construction of non-obvious synthetic modules to program cell function. Given recent advances that have uncovered the prominent role of additional cellular regulators, including regulatory RNAs [23] and prions [24], the field of synthetic biology might be well served to include such crucial regulatory components, which have been shown to directly affect cell phenotype.

One can foresee the development of an efficient technique for programmed cell function that combines an *in silico* evolution procedure of the type presented by François and Hakim with carefully controlled directed evolution of the 'most fit' architectures *in vivo*. This could be viewed as a circular process in which, based on our current level of understanding of gene regulatory network parts and interactions, an initial library of network components is evolved *in silico* to provide several architectures that exhibit desired behavior (Figure 2). These are then constructed and inserted into the chosen cell type. Appropriate selection criteria are applied, and

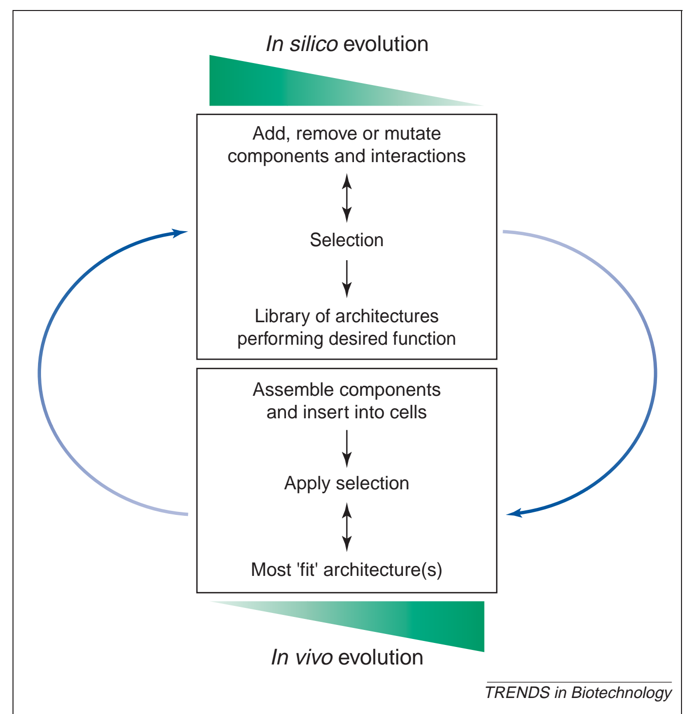

Figure 2. Integrated approach for creation of synthetic regulatory networks with desired functions. A library of gene-network architectures is designed by evolution *in silico* to perform a particular function. These are constructed *in vivo*, with each architecture subject to the same selective pressure based on desired function. Information gleaned from the best performing network architecture(s) is used to refine the computational algorithm.

the cells themselves select for the architectures that behave in the most robust manner. Herein lies the greatest experimental challenge: designing appropriate selection mechanisms that rely entirely on the cell population to choose the best architecture. Evolving networks within cells, instead of focusing on artificial networks in isolation, could provide the greatest potential for creating cells tailored to any particular function. In addition, by observing which architectures and individual component parts are most often 'chosen' by the cell, we gain tremendous insight into natural design principles. These favored components or designs can then be incorporated back into the *in silico* evolution algorithm for creation of subsequent architectures, bringing the process full circle. By selecting this formula for the creation of programmed cells, researchers might hasten the growing field of synthetic biology [25].

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Letter

Double humanized yeast makes hydrocortisone

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Hydrocortisone for use as an anti-inflammatory treatment for rheumatoid arthritis has been manufactured over the past fifty years using fungi such as *Rhizopus arthopus* or *Aspergillus niger*. These achieve the necessary step of β-hydroxylation, while *Cochliobolas lunatus* was relied upon for the α-hydroxylation.

Now total biosynthesis of hydrocortisone has been accomplished in recombinant DNA yeast [1,2] (baker's yeast; *Saccharomyces cerevisiae*) grown on a simple carbon source. The outstanding achievement was the humanization of the yeast cells by incorporation of cytochrome P (CYP) 450 *cyp* genes. These *cyp* genes were for human CYP11B1 and CYP21A1 and bovine CYP17A1 (these gene constructs were achieved using complimentary DNA). This manufacturing process for hydrocortisone [2] is a notable achievement that justifies fully the enabling biotechnology developed with baker's and brewers' yeast, throughout the previous century.

A GM yeast has also been developed with the potential for the direct fermentation of starch by amyloglucosidase (glucamylase). Otherwise, pre-degraded wheat starch or cornstarch can be used as brewing adjuncts to supplement

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