Hacking DNA copy number for circuit engineering

Feilun Wu & Lingchong You

DNA copy number represents an essential parameter in the dynamics of synthetic gene circuits but typically is not explicitly considered. A new study demonstrates how dynamic control of DNA copy number can serve as an effective strategy to program robust oscillations in gene expression circuits.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.

Variation in DNA copy number is a complex and widespread phenomenon (**Fig. 1a**). It often leads to altered gene expression that can be associated with human diseases such as cancer and developmental disorders[1]. On the unicellular level, this variation also has a critical role in microbial physiology. Yeast can respond to nutrient limitation by altering DNA copy number to modulate fitness[2]. In quickly growing bacteria, the copy number of a chromosomal gene decreases with its distance from the replication origin. Depending on the context, this property can act as an intrinsic mechanism to control the network dynamics underlying cell fate decisions[3], a parameter to be accounted for in quantitative analysis of noise[4] or a dynamic signature to enable quantification of bacterial growth in mixed populations[5]. In this issue, Jeff Hasty and colleagues demonstrate that rational control of DNA copy number is an effective strategy to program robust oscillations in bacterial colonies[6].

Engineering copy number control

It is well recognized that DNA copy number is a critical factor in controlling gene expression. When producing recombinant proteins, high DNA copy numbers allow for high gene expression levels, which can be detrimental to the cell as a result of elevated transcriptional and translational burden. This limitation can be alleviated by using plasmids with inducible amplification of copy number[7], such that the genes of interest are only highly induced under the appropriate condition. Despite having an important role in the dynamics of gene circuits, DNA copy number is typically not accounted for in modeling-mediated design and optimization of gene circuit function. The work by Baumgart *et al.*[6] represents the first attempt to program oscillations by rational control of plasmid copy number.

Numerous studies have demonstrated programming of oscillations in single cells or in cell populations using gene circuits[8]. The essential requirements for generating oscillations are often achieved by controlling the strengths of regulatory elements (for example, promoters and ribosome-binding sites) or the stability of interacting molecules[9]. In one circuit, the generation of oscillations critically depended on dynamic change in DNA copy number, which, however, was due to unintended variation in plasmid copy number by cell physiology[10].

The circuit described by Baumgart *et al.*[6] consists of an activator plasmid and a repressor plasmid (**Fig. 1b**). The activator plasmid encodes a quorum-sensing module, which drives its own expression as well as that of an endonuclease encoded by the repressor plasmid. The endonuclease cleaves the activator plasmid by recognizing a specific target sequence, thereby establishing a negative-feedback loop based on reduction of activator plasmid copy number. This copy number reduction is reversible upon removal of the inducing agent, creating tight temporal control. The resulting oscillatory signal is generated by a fluorescent protein that is encoded by the activator plasmid.

Coupling negative feedback with positive feedback is a strategy to increase the robustness of oscillations[9]. The authors also engineered quorum-sensing-mediated amplification of the repressor plasmid to investigate its effect on circuit dynamics. This was accomplished by induced overexpression of RNAII, an antisense RNA that has a role in plasmid replication. Indeed, the oscillations generated by this modified circuit were robustly maintained even with increased culturing chamber size, where the unmodified circuit only generated low-amplitude, irregular oscillations. This finding suggests that control of repressor plasmid copy number also modulates the robustness of circuit behavior across different culturing conditions.

Coupled with DNA copy number control, the quorum-sensing module is critical for both establishing feedback and synchronizing oscillations across a population through the collective production and sensing of a small, diffusible quorum-sensing signaling molecule[11]. However, an implicit factor of

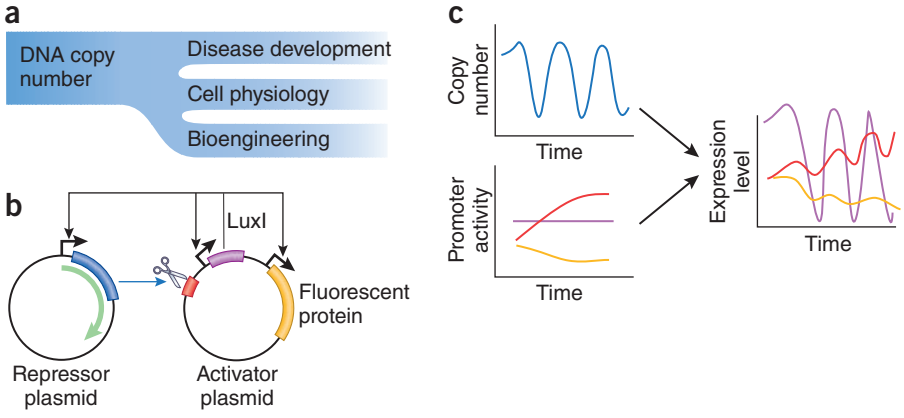

Figure 1 Dynamic DNA copy number control for programming cellular functions. (**a**) Implications of DNA copy number variation. (**b**) Circuit logic. The green arrow represents copy number amplification. LuxI is a part of the quorum-sensing module that produces quorum-sensing signals. (**c**) Dynamic DNA copy number control provides global regulation for genes (warm colors) that are independently modulated by different promoters.

Feilun Wu and Lingchong You are in the Department of Biomedical Engineering, Duke University, Durham, North Carolina, USA. Lingchong You is also in the Department of Molecular Genetics and Microbiology, Duke University School of Medicine and the Center for Genomic and Computational Biology, Duke University, Durham, North Carolina, USA.
e-mail: you@duke.edu

this coupling is the interplay between abundance of the quorum-sensing transcription factor and the number of transcription factor binding sites dictated by DNA copy number. This interplay may confound the design of DNA copy number control to regulate gene expression.

An expanded control toolkit

Transcriptional or translational regulation of gene expression has been the dominant control element used in gene circuits. However, there is still a lack of well-characterized components, such as orthogonal and compatible promoters. Rational control of DNA copy number represents a complementary strategy to current approaches. Baumgart *et al.*[6] leveraged an endonuclease and antisense RNA to achieve control of plasmid copy number. Alternatively, this can be accomplished by placement of genes in the chromosome to hijack different plasmid replication mechanisms[12]. Coupled with transcriptional or translational regulation, rational design of DNA copy number adds another layer of control that can increase the dynamic range and modulate the transcriptional cooperativity of gene expression.

A distinctive advantage of plasmid copy number control is that it enables coordinated regulation of expression of multiple genes. These genes can be further independently modulated by different regulatory elements (**Fig. 1c**). If we draw a parallel between genetic circuits and computer programs, the function encoded by a plasmid can be considered as a subroutine; regulation of plasmid copy number is equivalent to adding a global parameter when calling the subroutine. In engineered circuits, this feature may allow effective coordination of the expression of multiple effector genes. This capability is also potentially useful for controlling clusters of genes for metabolic engineering applications[13] or for probing certain aspects of cellular physiology, such as multidrug resistance[14] or biofilm development[15].

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Sebat, J. *et al. Science* **305**, 525–528 (2004).
2. Gresham, D. *et al. Proc. Natl. Acad. Sci. USA* **107**, 18551–18556 (2010).
3. Narula, J. *et al. Cell* **162**, 328–337 (2015).
4. Elowitz, M.B., Levine, A.J., Siggia, E.D. & Swain, P.S. *Science* **297**, 1183–1186 (2002).
5. Korem, T. *et al. Science* **349**, 1101–1106 (2015).
6. Baumgart, L., Mather, W. & Hasty, J. *Nat. Genet.* **49**, 1282–1285 (2017).
7. Friehs, K. *Adv. Biochem. Eng. Biotechnol.* **86**, 47–82 (2004).
8. Cameron, D.E., Bashor, C.J. & Collins, J.J. *Nat. Rev. Microbiol.* **12**, 381–390 (2014).
9. Cao, Y., Lopatkin, A. & You, L. *Nat. Struct. Mol. Biol.* **23**, 1030–1034 (2016).
10. Marguet, P., Tanouchi, Y., Spitz, E., Smith, C. & You, L. *PLoS One* **5**, e11909 (2010).
11. Garg, N., Manchanda, G. & Kumar, A. *Antonie Van Leeuwenhoek* **105**, 289–305 (2014).
12. Nordström, K. & Dasgupta, S. *EMBO Rep.* **7**, 484–489 (2006).
13. Rutledge, P.J. & Challis, G.L. *Nat. Rev. Microbiol.* **13**, 509–523 (2015).
14. Slager, J., Kjos, M., Attaiech, L. & Veening, J.W. *Cell* **157**, 395–406 (2014).
15. Stewart, P.S. & Franklin, M.J. *Nat. Rev. Microbiol.* **6**, 199–210 (2008).

The osteoarthritis and height *GDF5* locus yields its secrets

Guillaume Lettre

A new study reports molecular characterization of the *GDF5* locus, which is associated with osteoarthritis risk and adult height in humans. This study provides evidence of positive selection for short stature at *GDF5* in modern humans, as well as in archaic Neandertals and Denisovans.

This story begins back in 2007–2008 with the identification of SNPs strongly associated with osteoarthritis risk[1] and adult height[2] in humans. For both phenotypes, the most significant SNPs are near *GDF5* (growth differentiation factor 5), which encodes a protein related to the bone morphogenetic protein (BMP) family within the transforming growth factor (TGF)-β superfamily. *GDF5* is expressed in cartilage and developing joints and bones. Notably, *GDF5* mutations in mice and humans lead to skeletal defects, shorter bones and stature, and increased osteoarthritis risk. Given these data, *GDF5* represents an ideal candidate gene for the osteoarthritis and height association signals detected at this locus. However, the precise molecular mechanisms by which these SNPs modulate *GDF5* remained unknown. In a new study, David Kingsley, Terence Capellini and colleagues combine elegant transgenic experiments in mice with population genetic analyses in humans to identify a *GDF5* enhancer that harbors a strong candidate causal SNP under positive selection[3].

Guillaume Lettre is at the Montreal Heart Institute and the Faculté de Médecine, Université de Montréal, Montreal, Quebec, Canada.
e-mail: guillaume.lettre@umontreal.ca

Fine-mapping in transgenic mice

Genome-wide association study (GWAS) results are enriched for SNPs that map to noncoding regions that regulate gene expression, such as enhancers[4,5]. Postulating that the causal SNP at the *GDF5* locus might be a regulatory, noncoding variant, Capellini *et al.*[3] set out to identify enhancers that drive *GDF5* expression in the growth plates of long bones. They introduced large fragments of human DNA encompassing *GDF5* as well as its upstream or downstream regulatory sequences into mice to create mouse models of *GDF5* expression; these fragments also included a *lacZ* reporter gene to visualize *GDF5* expression patterns. Whereas the upstream sequence drove gene expression in joints and digits, the downstream sequence was required for expression in the growing ends of long bones. Furthermore, only *GDF5* fragments including the downstream sequence were able to rescue the phenotype of shorter long bones observed in *Gdf5*-null mice. These results argue that the sequence responsible for *GDF5* expression in growth plates is located downstream of the gene. Transgenic experiments with mice carrying smaller fragments of the human locus allowed the investigators to narrow down the regulatory element to a 2.5-kb enhancer—termed *GROW1*—located 69 kb downstream of *GDF5*. Importantly, CRISPR–Cas9-meditated deletion of the syntenic *Grow1* sequence in the mouse genome reduced *Gdf5* expression in growing bone ends and caused shorter long bones, thus confirming the regulatory role of this sequence *in vivo*.

On the basis of data from the 1000 Genomes Project, the authors found that *GROW1* contains only one common variant, rs4911178, in linkage disequilibrium (LD) with SNPs associated with osteoarthritis and height.

© 2017 Nature America, Inc., part of Springer Nature. All rights reserved.