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.

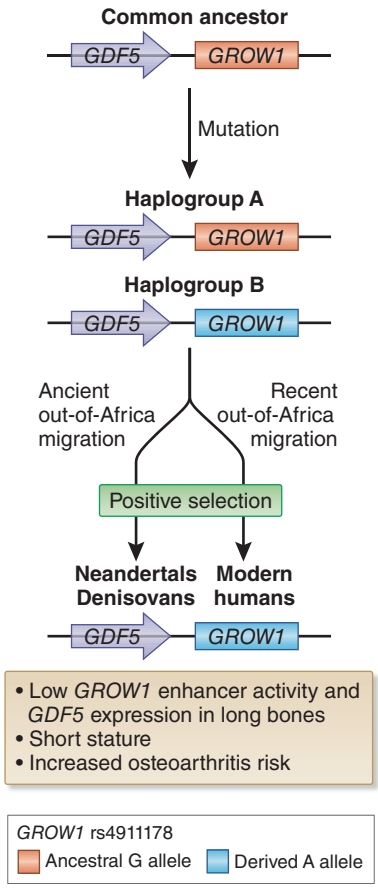

Figure 1 Model of positive selection for short stature at the *GDF5* locus in archaic and modern humans.

They tested the impact of allelic variation at rs4911178 on *GROW1* enhancer activity in transgenic mice and found that the derived A allele, which is associated with shorter height in humans, was associated with lower *GDF5* expression in long bones as compared to the ancestral G allele. This result was validated in a human chondrocyte cell line using a luciferase-based reporter assay. Altogether, these results confirm that *GROW1* is sufficient to drive *GDF5* expression in long bones and that the A allele at rs4911178 reduces *GROW1* enhancer activity.

Positive selection at *GDF5*

GDF5 and *GROW1* are located within a 130-kb haplotype that shows very high LD, a hallmark of positive selection[6,7]. Furthermore, the derived A allele at rs4911178 is frequent in Eurasians but rare in Africans and is also one of the top scoring variants in the haplotype to explain the signal of positive selection. Using data from the 1000 Genomes Project, the authors performed phylogenetic analyses and found two groups of haplotypes: group A was mostly present in African populations and included haplotypes that carried the ancestral G allele at rs4911178, whereas group B was frequent in Eurasians and included haplotypes that harbored the derived A allele at rs4911178 Surprisingly, when the team included DNA sequence data from the Neandertal and Denisovan genomes in the phylogenetic analyses, they found that these ancient *GDF5* haplotypes clustered within group B: both extinct hominins carried the short-stature-associated A allele at rs4911178 resulting in low *GROW1* activity. The most parsimonious—and fascinating—explanation for this observation, the authors argue, is that the short-stature-associated allele at rs4911178 in the *GDF5* locus was present in the last common ancestor of modern humans, Neandertals and Denisovans and has been under positive selection in archaic humans (Neandertals and Denisovans) and recent 'out-of-Africa' migrants (modern humans) (**Fig. 1**).

This study presents a convincing example of a regulatory causal SNP that explains a GWAS signal by disrupting the activity of a tissue-specific enhancer, adding this locus to only a handful of GWAS findings that have been characterized at this level of detail[8–10]. Some interesting questions still remain. Given the expression pattern driven by *GROW1*, it is unlikely that rs4911178 is the causal variant for osteoarthritis risk, so additional fine-mapping work on this long haplotype will likely be necessary to answer this riddle. Even for height, the situation might be more complex than initially thought. A large-scale GWAS found three independent SNPs associated with height in the *GDF5* locus[11]. One of these height association signals maps to *GROW1*, but the other two variants might affect uncharacterized *GDF5* enhancers. Further, rs4911178 is a strong expression quantitative trait locus (eQTL) for many genes in human tissues, including *GDF5*, but also *UQCC* and *CEP250* (ref. 12). It will be necessary to explore whether these genes, in combination with *GDF5*, might also influence osteoarthritis or stature. The work by Capellini *et al.*[3] is an impressive demonstration that thoughtful experiments will yield important insights in efforts to understand how genetic variation influences complex human phenotypes. It also serves as a reminder of the complexity of the task at hand, as researchers strive to characterize thousands of genetic associations identified by GWAS.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Miyamoto, Y. *et al. Nat. Genet.* **39**, 529–533 (2007).
2. Sanna, S. *et al. Nat. Genet.* **40**, 198–203 (2008).
3. Capellini, T.D. *et al. Nat. Genet.* **49**, 1202–1210 (2017).
4. Maurano, M.T. *et al. Science* **337**, 1190–1195 (2012).
5. Roadmap Epigenomics Consortium. *Nature* **518**, 317–330 (2015).
6. Voight, B.F., Kudaravalli, S., Wen, X. & Pritchard, J.K. *PLoS Biol.* **4**, e72 (2006).
7. Sabeti, P.C. *et al. Nature* **449**, 913–918 (2007).
8. Bauer, D.E. *et al. Science* **342**, 253–257 (2013).
9. Claussnitzer, M., Hui, C.C. & Kellis, M. *N. Engl. J. Med.* **374**, 192–193 (2016).
10. Musunuru, K. *et al. Nature* **466**, 714–719 (2010).
11. Wood, A.R. *et al. Nat. Genet.* **46**, 1173–1186 (2014).
12. GTEx Consortium. *Science* **348**, 648–660 (2015).