the TψC stem loop that builds out the corner. However, in archae, G15 is replaced after transcription by 7-cyano-7-deazaguanine through the action of archaeosine tRNA-guanine transglycosylase (ArcTGT). This enzyme "exchanges" G by breaking and resealing the glycosyl bond that joins the N9 position of the base to the C_1' carbon of the backbone ribose (Watanabe et al., 1997). The resulting modification is designated archaeosine. It retains the capacity to join together the two loops of the cloverleaf to make the corner of L form tRNA through bonding to C48. Plausibly, archaeosine provides further stability to the corner.

Not obvious is how ArcTGT achieves a posttranscriptional transformation that would seem to require breaking up the core and corner of the structure. Remarkably, Ishitani et al. (2003) were able to obtain a cocrystal of ArcTGT bound to a tRNA whose structure retains the L shape but has the D stem and the canonical core completely disrupted (Figure 1B). The entire region from U8 to U22 is not base-paired in the so-called λ-form. Remarkably, canonical parts of the classic cloverleaf are repaired in a new way, such that part of the D stem is now paired with the variable arm, while the remaining part of the D region is completely unpaired (Figure 1B). This conformation allows G15 to reach the active site of ArcTGT.

In contrast to the reorganization of the D loop and the variable loop in the λ-form, the conformation of the T loop is roughly the same as that of the canonical tRNA, despite the absence of the specific tertiary interactions between G18-G19 in the D loop with U55 (modified to ψ55) and C56 in the TψC-loop (Figure 1B). The "corner" of λ-form tRNA is strengthened in part by a new stacking interaction of A59 in the TψC loop over the G23:C48 pair. But other than this stacking interaction, the structure is stabilized by interactions with bound ArcTGT. From examining the pairing and stacking interactions of L- and λ-form tRNA, we doubt that the difference between the two forms is more than a few kcal mol^{-1}, an amount easily compensated by protein contacts.

That the structure of the minihelix domain in the λ-form is almost the same as in the L-form is of interest from an evolutionary standpoint. Several lines of evidence suggest that a minihelix-like RNA arose independently of the anticodon-containing domain and was the historical substrate for aminoacylation (Noller, 1993; Maizels and Weiner, 1994; Schimmel and Ribas de Pouplana, 1995). The minihelix itself is a substrate for specific aminoacylation by many aminoacyl tRNA synthetases through all three kingdoms of the tree of life. Evolutionary models for the development of tRNA start with a minihelix to which the second, anticodon-containing domain is added later (Di Giulio, 1992; Schimmel and Ribas de Pouplana, 1995; Rodin et al., 1996). Thus, the invariant conformation of the minihelix of λ-form tRNA may be a reflection of the evolutionary pathway of tRNA. For example, selective pressure for retention of aminoacylation may have developed an evolutionary context to limit in general perturbations of the minihelix. Pressure was then selectively applied to the second domain to bring in other functions or properties that are manifested in part through modifications.

Paul Schimmel and Koji Tamura
The Skaggs Institute for Chemical Biology
The Scripps Research Institute
Beckman Center, BCC379
10550 North Torrey Pines Road
La Jolla, California 92037

Selected Reading

Di Giulio, M. (1992). J. Theor. Biol. *159*, 199–214.

Ishitani, R., Nureki, O., Nameki, N., Okada, N., Nishimura, S., and Yokoyama, S. (2003). Cell *113*, this issue, 383–394.

Kim, S.H., Suddath, F.L., Quigley, G.J., McPherson, A., Sussman, J.L., Wang, A.H., Seeman, N.C., and Rich, A. (1974). Science *185*, 435–440.

Maizels, N., and Weiner, A.M. (1994). Proc. Natl. Acad. Sci. USA *91*, 6729–6734.

Motorin, Y., and Grosjean, H. (2001). tRNA Modification. In Encyclopedia of Life Sciences (Nature Publishing Group), 1–9.

Noller, H.F. (1993). On the origin of the ribosome: Coevolution of subdomains of tRNA and rRNA. In The RNA World, R. F. Gesteland and J. F. Atkins, eds. (Plainview, NY: Cold Spring Harbor Laboratory Press), 137–156.

Robertus, J.D., Ladner, J.E., Finch, J.T., Rhodes, D., Brown, R.S., Clark, B.F., and Klug, A. (1974). Nature *250*, 546–551.

Rodin, S., Rodin, A., and Ohno, S. (1996). Proc. Natl. Acad. Sci. USA *93*, 4537–4542.

Schimmel, P., and Ribas de Pouplana, L. (1995). Cell *81*, 983–986.

Watanabe, M., Matsuo, M., Tanaka, S., Akimoto, H., Asahi, S., Nishimura, S., Katze, J.R., Hashizume, T., Crain, P.F., McCloskey, J.A., and Okada, N. (1997). J. Biol. Chem. *272*, 20146–20151.

Coordinate Regulation of an Extended Chromosome Domain

Spitz et al. (2003 [this issue of *Cell*]) describe the properties of a novel *cis*-regulatory DNA element, the global control region (GCR), which regulates gene expression over distances of several hundred kilobases at the mouse *HoxD* complex. The GCR provides an explanation for the colinear genetic linkage and expression of individual *Hox* genes within developing limbs.

Gene expression is regulated by several different classes of *cis*-regulatory DNAs: enhancers, silencers, insulators, and the core promoter (Figure 1A). The promoter is the binding site for the RNA pol II transcription complex. Enhancers and silencers work over distances of several kilobases or more to stimulate or silence pol II function. Insulator DNAs prevent enhancers and silencers in one gene from inappropriately regulating a neighboring gene. Recent studies suggest that there may be a distinct class of *cis*-regulatory DNAs, "domain control elements," which coordinate the expression of linked genes over large chromosome domains.

A recent bioinformatics survey suggests that linked genes exhibit coordinate expression in the *Drosophila* genome (Spellman and Rubin, 2002). On average, some-

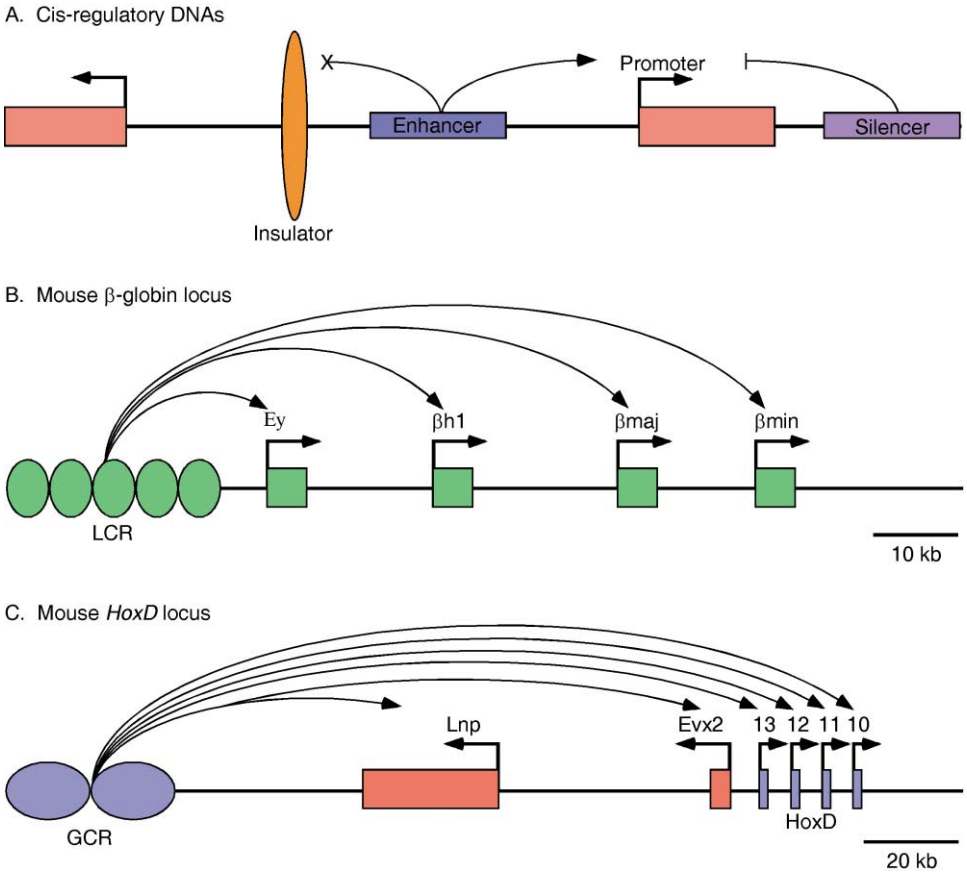

Figure 1. Domain Control Elements Coordinate Gene Expression over Long Distances

thing like 10–20 genes spanning a domain of 100–200 kb constitute a common domain. Genes within a given domain are subject to similar temporal regulation. These findings could be explained by the presence of higher order control elements. Each of the estimated 500–1000 domains in the *Drosophila* genome could contain a control element that coordinately regulates gene expression within that domain. Here, we review the evidence that the newly identified GCR functions as a domain control element, but we first summarize the properties of a related element, the locus control region (LCR) in vertebrate β-globin loci (for reviews see Bulger and Groudine, 1999; Fraser and Grosveld, 1998).

The LCR is required for optimal expression of globin genes, and several models have been proposed to explain how it works (Figure 1B). Most of these models invoke gene "competition," whereby individual promoters sequester the LCR and prevent the activation of the more distal genes within the β-globin locus. According to this model, the LCR is "trapped" on the first accessible promoter(s) it encounters, and thereby activates the *Ey* and β*h1* genes during early development. At later stages, sequence-specific repressors silence the embryonic promoters, releasing the LCR. Silencing of the proximal embryonic promoters permits the LCR to interact with the more distal genes and activate the expression of the β*maj* and β*min* genes in newborn mice.

The GCR regulates the spatial expression of *HoxD* genes in the developing limbs (Figure 1C). Genes close to the GCR exhibit higher levels of expression in distal regions of developing limbs than those located in more remote regions of the *HoxD* locus. Thus, *Hoxd11* and *Hoxd10* are expressed at lower levels than *Hoxd13* and *Hoxd12*. Deletions that remove the *Hoxd13* and *Hoxd12* genes cause augmented expression of *Hoxd10* in distal regions (Kmita et al., 2002). This overexpression of *Hoxd10* disrupts the normal patterning of the limbs.

While the LCR is specific for β-globin genes, the GCR is not restricted to the *HoxD* genes. The mouse *HoxD* locus maps near the *Evx2* gene, and the newly identified *Lunapark* (*Lnp*) locus. The GCR coordinates the expression of all of these genes. It is essential for the restricted expression of the *Lnp* and *Evx2* genes in the CNS, as well as the sequential expression of *HoxD* genes in the developing limbs.

The sequential activation of the *Ey*/β*h1* and β*maj*/β*min* genes in the globin locus, and the *Hoxd13*, *Hoxd12*, *Hoxd11*, and *Hoxd10* genes in the *HoxD* locus depends on the organization of these genes within their respective loci. The *Ey*/β*h1* globin genes are the first to be activated because they are located closest to the LCR, while the *Hoxd13* and *Hoxd12* genes are expressed at the highest levels in the distal limbs because they are closest to the GCR. However, gene competition is not

determined solely by genetic organization. For example, the *Ey*/β*h1* globin promoters might be "stronger" than the β*maj* and β*min* promoters, thereby ensuring their early activation by the LCR. Similarly, the *Hoxd13* and *Hoxd12* promoters might be stronger than the *Hoxd11* and *Hoxd10* promoters.

Promoter strength may be an intrinsic property of the core promoter. Perhaps the *Ey* and β*h1* promoters contain optimal binding elements for TFIID and RNA polymerase (e.g., TATA and INR), whereas the β*maj* and β*min* promoters contain suboptimal core elements. Alternatively, competition might depend on proteins that bind at promoter-proximal regions, just 5′ of the core promoter (Calhoun et al., 2002; Foley and Engel, 1992). Some of these proteins might help recruit distal enhancers, or stabilize enhancer-promoter interactions. Perhaps the first promoter-proximal binding proteins that appear during mouse development bind to the *Ey* and β*h1* promoter regions. Later in development, these proteins are lost and others appear that bind to the β*maj* and β*min* promoter regions. Altogether, gene competition is influenced by all of the aforementioned properties: genetic organization, the strength of the core promoter elements, and the presence of promoter-proximal binding proteins.

The GCR is not completely trapped by the *Hoxd13* promoter since the more distal *Hoxd12*, *Hoxd11*, and *Hoxd10* genes are also activated. Perhaps all of the *Hoxd* promoter-proximal binding proteins appear simultaneously during mouse development. As a result, each gene can be activated by the GCR, but those mapping in the 5′-most regions of *HoxD* locus are the most strongly activated due to their proximity to the GCR. In contrast, the temporal order of globin gene expression might depend on the sequential appearance of promoter-proximal binding proteins during development.

The GCR and LCR coordinate the regulation of gene expression over extended chromosome domains. An LCR-like element has also been implicated in the regulation of red and green pigment genes in the cone cells of the retina (Smallwood et al., 2002). It is currently unclear whether these regulatory DNAs are exceptional or represent a common feature of metazoan genomes.

Vincent C. Calhoun and Michael Levine
Department of Molecular and Cell Biology
Division of Genetics and Development
University of California, Berkeley
Berkeley, California 94720

Selected Reading

Bulger, M., and Groudine, M. (1999). Genes Dev. *13*, 2465–2477.

Calhoun, V.C., Stathopoulos, A., and Levine, M. (2002). Proc. Natl. Acad. Sci. USA *99*, 9243–9247.

Foley, K.P., and Engel, J.D. (1992). Genes Dev. *6*, 730–744.

Fraser, P., and Grosveld, F. (1998). Curr. Opin. Cell Biol. *10*, 361–365.

Kmita, M., Fraudeau, N., Herault, Y., and Duboule, D. (2002). Nature *420*, 145–150.

Smallwood, P.M., Wang, Y., and Nathans, J. (2002). Proc. Natl. Acad. Sci. USA *99*, 1008–1011.

Spellman, P.T., and Rubin, G.M. (2002). J Biol *1*, 5.

Spitz, F., Gonzalez, F., and Duboule, D. (2003). Cell *113*, this issue, 405–417.