Registering the reference gene expression from hundreds of embryos permitted the registration of the second gene's expression, producing an average 3D expression map for gene X or Y. Although the in situ hybridizations of gene X and Y were conducted independently, their expression patterns can be overlaid and compared as their average expression patterns are mapped to a common model (the virtual embryo). This allows spatial multiplexing in silico at an almost infinite scale.

The authors performed this expression mapping for 95 genes during a 50 min interval prior to gastrulation—an impressive body of work. The results are very exciting: 3D virtual images that look almost identical to simultaneous in situs performed in the same embryo. It is very difficult to quantitate how similar "almost identical" is. The authors assessed this by comparing the variability in expression of two genes (*giant* and *eve*) measured in the same embryo (a standard double in situ hybridization) to that inferred by their registration method for the two genes imaged in different embryos. The variability in expression was measured by grouping nuclei that have similar expression levels of *giant* into bins and then looking at the average expression levels of *eve*. The maximum standard deviation of *eve* expression in all bins was 0.21 for the "real" double in situs and 0.3 in the virtual embryo, while the maximum expression levels of *eve* and *giant* deviate by <7% between the two approaches. By these two criteria, the virtual embryo provides an accurate average quantitation of gene expression in 3D.

Many studies have tried to reconstruct gene regulatory networks based on expression data under the assumption that a transcription factor and a target gene will be temporally coexpressed for some period of time or have a small temporal shift in their expression. These studies are primarily based on microarray profiling data, as this has been the only global quantitative data available. The extraction of relative gene expression data using virtual embryos opens a new avenue for inferring regulatory connections at a cell-based level. Given the much higher spatial resolution, these data, in combination with other data such as transcription factor binding site occupancy, should vastly improve the predictive power of regulatory models. There is a clear need to move toward a quantitative protein atlas of transcription factor expression, integrated with quantitative measures of their target genes' mRNA expression. The computational methods developed by Fowlkes et al. (2008) are an important first step in this direction.

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Together We Stand: Genes Cluster to Coordinate Regulation

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Although most eukaryotic genomes lack operons, occasionally clusters of genes are discovered that are related in function. Now, a metabolic operon-like gene cluster has been described in *Arabidopsis thaliana* that is needed for triterpene synthesis.

Traditionally, genes were considered to be randomly distributed in eukaryotic genomes, in stark contrast with prokaryotes, where unrelated genes with related functions are clustered in so-called operons. However, in the last few years, genomic data reveal that also in eukaryotic genomes, genes can be physically clustered. The best known example is probably *Caenorhabditis elegans*, where almost 15% of the genes are organized in polycistronic operons, but also in yeast,

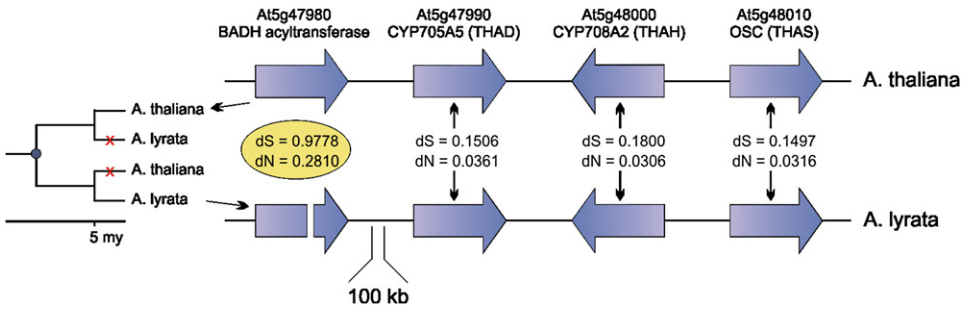

Figure 1. Triterpene Gene Clusters in *Arabidopsis thaliana* and *A. lyrata*

While the structure of three out of four genes is the same in both species, the structure of the *A. lyrata* homolog of At5g47980 is different (indicated by a split gene). Furthermore, these latter homologs in both species differ much more than the others (see dS and dN values), which might suggest paralogy rather than orthology (see tree). Noteworthy is also a large insertion of >100 kb (mostly transposons) in the cluster in *A. lyrata,* which is missing in *A. thaliana*. dS, number of synonymous substitutions per synonymous site; dN, number of nonsynonymous substitutions per nonsynonymous site.

metabolic clusters have been observed (Hurst et al., 2004; Wong and Wolfe, 2005; Yi et al., 2007). The identification of clusters of genes with similar functions or involved in the same pathway or biological process raises intriguing questions about the molecular mechanisms and evolutionary pressures responsible for such self-organization. It is generally considered that this kind of spatial genomic organization allows for tightly coordinated gene expression and for genetic linkage of functionally related genes. Therefore, the organization of functionally related genes in clusters is expected to have an evolutionary advantage for the organism.

In monocot plants, some metabolic clusters have been uncovered, such as the one for benzoxazinoid biosynthesis in maize, diterpene biosynthesis in rice, and triterpene biosynthesis in oat (Gierl and Frey, 2001; Qi et al., 2004; Shimura et al., 2007). In a recent paper in *Science*, Field and Osbourn (2008) showed the formation of a metabolic gene cluster for triterpene biosynthesis in the dicot *Arabidopsis*. The authors describe the identification and functional characterization of a metabolic pathway that is responsible for the synthesis and further modification of a triterpene saponin named thalianol. Triterpenes are secondary metabolites, which usually protect plants against pests and diseases. One-quarter of all *Arabidopsis* genes is predicted to be involved in secondary metabolism, but generally these metabolic genes are not clustered in plants, with the previously mentioned exceptions of diterpene, triterpene and benzoxazinoid biosynthesis clusters in rice, oat and maize, respectively. Field and Osbourn (2008) identified a genomic region that contains four contiguous genes encoding a 2,3 oxidosqualene cyclase (OSC), two CYP450s, and a BADH acyltransferase (see Figure 1). Interestingly, the oat "avenacin" cluster is also composed of a monocot-specific OSC gene, a monocot-specific CYP450, and a monocot-specific acyltransferase, among other genes, such as glycosyltransferases. Using null insertion mutants, RNA interference (RNAi) knockdowns, and GC-MS analysis, Field and Osbourn could demonstrate that the *Arabidopsis* gene cluster indeed encodes a pathway for the synthesis and further modification of thalianol. However, they could not detect the product that is predicted to be modified by the fourth gene of the cluster, the acyltransferase BADH.

It has been proposed that one of the consequences of the clustering of genes with the same function or involved in the same pathway is that their expression is tightly coordinated at the chromatin level (Hurst et al., 2004), and, indeed, the expression of all four *Arabidopsis* genes in the thalianol cluster is highly correlated ($r = 0.86$). Furthermore, all four genes are predominantly expressed in the root epidermis, as is the case with the genes in the oat avenacin cluster. In addition, the four genes of the thalianol cluster have marked histone H3 lysine 27 trimethylation, while this is not the case for their immediate neighboring genes.

Biochemical pathways usually do not form isolated networks that run in parallel, but on the contrary are highly interconnected (Pichersky and Gang, 2000). Changes in expression levels and tissue distribution of some enzymes may result in ectopic expression and disruption of other established tissue-specific pathways. This interference may happen because a small number of enzyme groups have considerably expanded through gene duplication. The duplicates are optimized for a certain substrate, but they can still have an effect on other similar, but noncognate, substrates. Indeed, Field and Osbourn (2008) demonstrate that the ectopic overaccumulation of intermediate products of the thalianol pathway in above-ground tissues led to severe dwarfing, whereas elevated levels of the same products resulted in longer roots than the wild type. Also, in the case of the oat triterpene avenacin, mutants of *sad3* or *sad4* glycosylation have aberrant root morphology. Evidently, tight regulation of these two similar triterpene pathways is required in order to ensure controlled development, and this is achieved by the formation of tightly regulated gene clusters.

The question remains whether the root-specific epidermal triterpene gene clusters in oat and *Arabidopsis* have arisen by chance, horizontal gene transfer, or convergent evolution, or do they, on the contrary, share common ancestry? Statistical tests show that the probability of finding five loci of saponin biosynthesis within 3.6 cM in oat, assuming a random distribution of genes, is extremely low (Qi et al., 2004). Although no equivalent analysis has been performed for the thalianol gene cluster in *Arabidopsis*, it seems that coincidental clustering also here can be excluded. Triterpene saponins are widely found in eudicots,

whereas in cereals and grasses, they do not seem to be present, with the exception of the Avena (oat) genus. A series of DNA and RNA blot analyses in several cereals did not identify any orthologs of the avenacin Sad1 gene (Qi et al., 2004). Of note, oat possibly diverged from the eudicots about 150 million years ago. It has been demonstrated that extensive gene loss of secondary metabolite synthases in microorganisms could mistake a case of common ancestry for horizontal gene transfer or convergent evolution (Rantala et al., 2004). However, phylogenetic analyses of the avenacin and thalianol pathways show that the genes of each pathway are monocot and eudicot specific, respectively. Therefore, Field and Osbourn (2008) exclude horizontal gene transfer from bacteria or other organisms. Unless we are dealing with a case of rampant gene duplication, rapid neofunctionalization, and gene losses of the ancestral genes, which actually might not be so far-fetched for secondary metabolism, the most reasonable scenario is convergent evolution and repeated de novo synthesis of the avenacin and thalianol gene clusters, as suggested by the authors. Cases of convergent evolution have been reported before for regulatory networks involved in animal development (Amoutzias et al., 2004) or for the formation of pathways of secondary metabolism (Pichersky and Gang, 2000). Nevertheless, the birth of clusters of functionally related genes with tightly coordinated expression seems a rather rare event in eukaryotic genomes, but, undoubtedly, upcoming plant genomes will shed more light on fundamental questions regarding the structure and evolution of such gene clusters (see for example Figure 1).

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Glucose Restriction: Longevity SIRTainly, but without Building Muscle?

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The two metabolic sensors AMPK and SIRT1 take center stage as Fulco et al. reveal, in this issue of *Developmental Cell*, the signaling mechanism by which low glucose prevents the correct development of the myogenic program. These observations may hold some therapeutic promise against muscle wasting.

One of the most amazing features of skeletal muscle is its high plasticity, enabling it to respond to changes in activity, injury, or degeneration. This plasticity is largely due to muscle stem cells, better known as satellite cells, residing beneath the basal lamina of adult skeletal muscle, closely juxtaposed against the muscle fibers (Le Grand and Rudnicki, 2007), which have the ability to modulate muscle growth and differentiation. Satellite cells in adult skeletal muscle are normally quiescent, but proliferation and differentiation of their descendant cells can be activated by diverse forms of stress, thereby playing an essential role in muscle regeneration, muscle hypertrophy, and postnatal muscle growth.

Despite the astonishing advance during the last few decades in our understanding of the process of myogenesis (reviewed in Le Grand and Rudnicki, 2007), the molecular mechanisms regulating the differentiation of myogenic stem cells are still unclear. In this issue of *Developmental Cell*, a new report from Fulco et al. (2008) examines the effects of nutrient availability on myogenic differentiation. Interestingly, the authors find that restricted glucose availability prevents myogenesis. Furthermore, the authors identify AMP-activated protein kinase (AMPK)—a master switch of anabolic versus catabolic processes—as a key sensor of low glucose levels during myogenesis. The concept that low glucose levels leads to the