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An oscillatory network controlling self-renewal of skeletal muscle stem cells

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Abstract

The balance between proliferation and differentiation of muscle stem cells is tightly controlled, ensuring the maintenance of a cellular pool needed for muscle growth and repair. Muscle stem cells can proliferate, they can generate differentiating cells, or they self-renew to produce new stem cells. Notch signaling plays a crucial role in this process. Recent studies revealed that expression of the Notch effector HES1 oscillates in activated muscle stem cells. The oscillatory expression of HES1 periodically represses transcription from the genes encoding the myogenic transcription factor MYOD and the Notch ligand DLL1, thereby driving MYOD and DLL1 oscillations. This oscillatory network allows muscle progenitor cells and activated muscle stem cells to remain in a proliferative and 'undecided' state, in which they can either differentiate or self-renew. When HES1 is downregulated, MYOD oscillations become unstable and are replaced by sustained expression, which drives the cells into terminal differentiation. During development and regeneration, proliferating stem cells contact each other and the stability of the oscillatory expression depends on regular DLL1 inputs provided by neighboring cells. In such communities of cells that receive and provide Notch signals, the appropriate timing of DLL1 inputs is important, as sustained DLL1 cannot replace oscillatory DLL1. Thus, in cell communities, DLL1 oscillations ensure the appropriate balance between self-renewal and differentiation. In summary, oscillations in myogenic cells are an important example of dynamic gene expression determining cell fate.

Introduction

Skeletal muscle grows during development and in postnatal life. Moreover, the adult muscle has the capacity to regenerate after injury. The cellular sources for skeletal muscle growth in fetal and postnatal development are muscle progenitor cells, whereas muscle stem cells (MuSC) allow regeneration in the adult. MuSC derive from myogenic progenitor cells in development (hereafter called muscle progenitors or progenitor cells). Like progenitor cells, they express *Pax7* and/or *Pax3* [1-8]. Muscle progenitor cells proliferate during development and in the postnatal period when they generate differentiating cells for muscle growth, and they also self-renew to replenish their own numbers. In the adult, MuSC acquire quiescence, but they are activated to proliferate in response to muscle injury, which was extensively analyzed in mice. Activated MuSC can either generate new muscle fibers for repair, or they replenish the stem cell pool that can be used for further muscle regeneration [9-11].

Adult skeletal muscle stem cells represent a small cell population and were originally defined in the frog as satellite cells based on their anatomical location between the basal lamina and plasma membrane of the myofiber [12]. These cells can be identified by the expression of marker genes. Pax7 expression is the most commonly used marker to identify muscle stem cells experimentally, and we use in this review the term MuSC for Pax7+ cells in the muscle observed *in vivo*, regardless whether these are quiescent or activated, or for isolated MuSC that were cultured for short time. In mice, Pax7+ cells take up the position between the basal lamina and the fiber, that is referred to as the ‘niche’, late in fetal development when a matrix begins to form around the muscle fiber [13]. The niche provides a specialized microenvironment in which MuSC receive signals that keep them in an undifferentiated state and maintain their quiescence over long periods of time [14-16]. Accordingly, the myogenic differentiation factors MYOD, MYF5 and MRF4 are not produced by quiescent MuSC in adult mice, and are only present when the cells become activated and enter the cell cycle. Sustained expression of these factors induces myogenic differentiation, not only in myogenic cells (myogenic cells are defined here as muscle progenitors, MuSC and cells of the myogenic cell line C2C12) but also in fibroblasts and adipocytes [17-19]. *In vivo, MyoD*, *Myf5* and *Mrf4* can compensate for each other, although mouse mutants for these genes have subtle and distinct phenotypes [20-22].

Canonical Notch signaling regulates the balance between proliferation and differentiation in muscle progenitors and MuSC, thus playing an important role in myogenesis. The mechanism of Notch signaling is highly conserved in evolution, and controls development and tissue maintenance in invertebrate and vertebrate organisms. Notch ligands (e.g. Delta and Jagged) presented by the signal-sending cell activate Notch receptors on the neighboring signal-receiving cell, which results in the cleavage of the Notch intracellular domain (NICD). NICD then translocates to the nucleus where it interacts with RBPJ, the principal transcriptional mediator of Notch signaling, to induce Notch target genes [23, 24]. A further prerequisite for Notch signaling is that the ligand needs to be presented by a cell or be bound to another surface like a culture dish, implying that soluble ligands cannot efficiently activate the Notch receptors [25]. It was suggested that interactions between the ligands and Notch are followed by endocytosis of the ligand-receptor complex, which exerts a pulling force on the receptor [26]. This facilitates Notch cleavage, the release of NICD, and the activation of Notch target genes. Notch signaling regulates a number of target genes in myogenic cells of mice, including those encoding extracellular matrix proteins [13, 16, 27]. The best-known Notch target genes encode transcriptional repressors of the HES/HEY family of bHLH proteins. *Hes/Hey* genes are induced by Notch signals in many organisms and cell types, including myogenic cells [28, 29].

The first function of Notch discovered in mammalian cells was the control of differentiation. Forced Notch activation suppresses myogenic differentiation in mice and chicken [24, 30-37]. Conversely, genetic ablation of Notch signaling in mice, more specifically the ablation of *Dll1* or *RBPj* in development, or the ablation of *Dll1*, *Rbpj*, *Hey/Heyl* or *Hes1* in the adult, results in MYOD upregulation and premature myogenic differentiation [13, 37-42]. Thus, due to uncontrolled differentiation, the pool of muscle progenitors and MuSC is depleted quickly and, as a consequence, muscle growth and regeneration are impaired. The fact that mutations of Notch target genes like *Hes/Hey* have similar phenotypes as the mutation of *Rbpj* indicates that *Hes/Hey* mediate the Notch effects on myogenic differentiation, in particular the suppression of *MyoD*. Interestingly, work done on the developing muscle shows that the drastic deficit caused by ablation of *Rbpj* is largely rescued by the mutation of

MyoD [13]. Thus, in the absence of Notch signals, uncontrolled *MyoD* expression causes premature myogenic differentiation that ultimately results in the depletion of the progenitor pool.

In addition to regulating self-renewal and differentiation, MuSC also require Notch signaling to maintain their quiescence. Thus, ablation of *Rbpj* or Notch target genes *Hey/Heyl* in MuSC of adult mice results in a loss of quiescence [13, 37-41]. In accordance, recent systematic analyses indicate that quiescent MuSC in their niche express very high levels of Notch target genes which are reduced upon activation [9]. This is preceded by the appearance of a mitogen-activated protein kinase (MAPK or MAP kinase) or stress kinase gene signature. Thus, high Notch and low MAPK signaling activity are hallmarks of quiescent MuSC. In comparison, Notch signaling is lower but still active in proliferating MuSC, whereas MAPK activity is increased [9].

An oscillatory network driven by Notch signaling

Genes encoding the HES/HEY family of transcription factors are strongly induced by Notch signaling and function frequently as crucial mediators of Notch signals. HES/HEY proteins function as potent repressors of gene transcription and bind to N-box sequences in the genome as homo- or heterodimers [28, 29, 43, 44].

Among the members of the mammalian HES/HEY family, HES1 is best studied [29, 44]. Binding of HES1 to N-boxes upstream of the *Hes1* promoter represses its own transcription and results in a negative feedback-loop [44]. Together, the negative feedback, the short half-lives of *Hes1* mRNA and HES1 protein, and the delay between transcript and protein production results in oscillatory expression of HES1 ([29]; see also below). The oscillatory expression of *Hes* genes can be observed in several cell types, notably among them activated MuSC [39]. Oscillatory expression of Notch signaling components was first discovered in the presomitic mesoderm where it controls somitogenesis [45], and also occurs in neuronal and pancreatic stem cells [46, 47]. In most cell types, these oscillations occur in an asynchronous manner (myogenic, neuronal, pancreatic cells), meaning that neighboring cells display oscillations that are out of phase or phase shifted. In contrast, oscillations in

the presomitic mesoderm are synchronous and neighboring cells oscillate in the same phase.

While oscillations are conserved in vertebrates, oscillatory periods are not. The best example of this is HES7, whose oscillatory activity in the presomitic mesoderm has been studied in many species. HES7 oscillates in the presomitic mesoderm with a period of 2-3 hours in mice [48]. In human presomitic mesoderm cells derived from induced pluripotent stem cells, the oscillatory period of HES7 is considerably longer (5 hours), whereas the period in the chick presomitic mesoderm is slightly shorter (1.5 hours) [45, 49, 50]. Thus, oscillatory gene expression has been observed in many vertebrate species, but as of yet has not been described in Drosophila. The different oscillatory periods observed in different species are due to species-specific biochemical reaction velocities [51, 52]. In accordance, the oscillatory periods in the presomitic mesoderm become longer when the temperature is reduced [53]. In summary, compared to the oscillations regulated by the circadian clock or the cell cycle, the oscillatory period of HES proteins are short. The oscillatory expression of *Hes/Hey* genes results in the rhythmic repression of target genes, and if the transcripts and proteins of these targets are unstable, their expression can also oscillate. Thus, oscillatory expression of HES/HEY can drive an entire oscillatory network.

Tools for the analysis of dynamic gene expression in MuSC

Methods such as *in situ* hybridization and immunostaining rely on fixation and provide a temporal snapshot of transcript and protein expression, severely limiting their use for the analysis of oscillatory expression dynamics. Time-lapse recordings of a reporter make it possible to visualize the dynamics of gene expression. Due to the short period of oscillations, the reporter expression response must be rapid and the reporter mRNA and protein must be unstable. In our work we used luciferase as a reporter because luciferase is highly sensitive, has a wide dynamic range and lacks auto-luminescence, making the bioluminescence reporter ideal for live imaging of muscle tissue [54, 55]. Further, luminescence does not require light excitation, which prevents photo damage to the cells during time-lapse imaging [56, 57]. Additionally, luciferase immediately generates luminescence in the presence of ATP and its

substrate luciferin. In contrast, most fluorescent proteins take a few hours to properly fold and emit fluorescence [58, 59]. However, the detection of luciferase activity in individual cells requires sensitive equipment, particularly a highly sensitive camera. It should be noted that newly developed fluorescent proteins like Achilles or Venus possess shorter folding times, and destabilized variants have been successfully used to monitor oscillatory gene expression [50, 60]. These fluorescent proteins work well in cells and tissues in which autofluorescence is low.

We have observed oscillatory gene expression in MuSC that were derived from fetal, postnatal, adult or aged transgenic mice kept under different conditions: (i) MuSC that were cultured in a dish or as spheres (‘myospheres’), (ii) MuSC associated with a single muscle fiber (floating fiber cultures), and (iii) MuSC in a skeletal muscle biopsy or in tissue slices [38, 39]. Thus, oscillatory gene expression in MuSC is observed in a wide range of experimental settings, but once the cells begin to terminally differentiate and fuse, oscillations cease.

The oscillatory pacemaker HES1 drives MYOD oscillations

A first indication of the dynamic expression of HES1 in murine MuSC had been the markedly heterogeneous HES1 expression levels in activated MuSC *in vivo* or in freshly isolated MuSC cultured for short time. Subsequent analyses using a transfected reporter construct in which the *Hes1* promoter drives a short-lived firefly luciferase showed oscillatory bioluminescence. A direct test of whether the protein also oscillates was possible by imaging primary muscle MuSC from a transgenic mouse strain that expresses a HES1-luciferase fusion protein. Together, these experiments unambiguously demonstrated oscillatory *Hes1* mRNA and protein expression in MuSC [39].

In addition to the heterogeneity of HES1 protein, also MYOD protein levels were noted to be markedly heterogeneous in activated MuSC *in vivo* or in cell culture, which raised the possibility that also MYOD expression oscillates. This was directly tested using isolated MuSC from a mouse strain in which luciferase DNA was inserted in frame into the 3′ coding sequence of *MyoD*. Indeed, MYOD expression was found to oscillate in activated MuSC in culture, when they were associated with

myofibers, or in muscle explant culture. MYOD protein levels were previously reported to vary during the cell cycle. This was observed when cells of the C2C12 cell line were blocked in the cell cycle and subsequently released, resulting in cell cycle synchronization [61, 62]. However, in non-synchronized MuSC cultured for short periods, MYOD protein levels did not correlate with the cell cycle [39]. For instance, no correlation of a mitotic marker and MYOD/HES1 expression levels was observed. Further, MuSC in such cultures divided regardless whether MYOD oscillations were at peaks or troughs. Finally, when fiber-associated MuSC are cultured and become activated, their first division occurs after about 42 hours, but robust oscillations occurred at least 24 hours before the first division [39].

Interestingly, when MuSC were kept in differentiation medium, a period of sustained MYOD expression was observed prior to terminal differentiation and fusion [39]. In summary, HES1 and MYOD oscillate as long as MuSC remain in a proliferative state, and during in the oscillatory phase cells are ambivalent and can choose between self-renewal or differentiation. When oscillations cease and MYOD expression is sustained, the cells enter into terminal differentiation.

The oscillatory periods of HES1 and MYOD are similar. This is due to the fact that HES1 directly represses *MyoD*, thereby driving MYOD oscillations. Several lines of evidence support this statement. Foremost, MYOD oscillations were unstable when *Hes1* is ablated in mice, and the destabilized oscillations were observed under various conditions, e.g. in cultured MuSC, in activated MuSC associated with a single fiber, and cultured muscle biopsies containing activated MuSC. Additional experiments strengthened this notion: transfection of *Hes1* suppressed MYOD, and conversely, MYOD was up-regulated when *Hes1* is ablated. Finally, ChIP-PCR experiments showed that HES1 can control *MyoD* directly through several binding sites located in the *MyoD* promoter, the core enhancer [63], and a highly conserved but previously uncharacterized sequence 10.5 kb upstream of the transcription start site [39].

DLL1 and the oscillatory network

Dll1 is expressed in a salt and pepper pattern in the developing muscle and in MuSC in the regenerating muscle of mice. Detailed analysis indicates that activated MuSC (PAX7+/MYOD+) and differentiating (MYOG+) cells express *Dll1*, but not quiescent MuSC or myofibers [38, 64]. Specific ablation of *Dll1* in adult MuSC changed the behavior of activated MuSC, i.e. decreased self-renewal and increased differentiation propensity. Thus, DLL1 produced by MuSC suppressed differentiation of neighboring MuSC [38]. This is reminiscent of the mechanism of lateral inhibition first described in invertebrates [65, 66].

DLL1 expression dynamics was studied using an allele encoding a DLL1-luciferase fusion protein (Dll1^{luc}), which showed that DLL1 is expressed in an oscillatory manner in activated MuSC (Fig. 1A). DLL1 oscillations were observed in single cells, as well as in cell communities. The oscillatory period was again 2-3 hours, and thus similar to the oscillatory period of MYOD and HES1, indicating that DLL1 oscillations are controlled by oscillatory expression of MYOD and/or HES1. Indeed, ablation of *Hes1* interfered with DLL1 oscillations and increased overall DLL1 expression levels, whereas ablation of *MyoD* reduced expression levels but allowed oscillations (Fig. 1B). Thus, HES1 is the pacemaker that drives the oscillatory network encompassing DLL1 and MYOD, whereas MYOD controls the robustness of DLL1 expression. The enhancer that drives the oscillatory expression was first identified in ChIP-Seq experiments [67] and is located in a *Dll1* intron. The enhancer binds MYOD and HES1, and a fragment encompassing these HES1 and MYOD binding sites was sufficient to convey oscillatory expression of a destabilized luciferase expression construct in cultured murine MuSC [38].

In summary, oscillating HES1 in myogenic cells controls oscillatory expression of two target genes, *MyoD* and *Dll1*. In this network, HES1 represses *Dll1*, *MyoD* and its own promoter, whereas MYOD regulates *Dll1* in a positive manner. Thus, the individual components regulate each other in an oscillatory network that functions in a cell-autonomous manner, resulting in oscillations of MYOD, HES1 and DLL1 proteins (Fig. 1C, D). Despite the fact that HES1 suppresses *MyoD* and *Dll1*, the oscillatory phases of MYOD and DLL1 proteins are shifted in respect to each other (Fig. 1D), which is due to differences in protein/mRNA stability and times needed for transcription and translation. During muscle development or regeneration, myogenic

cells are present in communities in which the cells contact each other. In such communities, DLL1 presented by the signal-sending cell induces the expression of HES1 the neighboring, signal-receiving cell. MYOD, HES1 and DLL1 oscillates in both cells, and the oscillatory phases of the proteins are shifted in the neighboring cells (Fig. 2A, an example of the shift in oscillations is shown for DLL1). It should be noted that the oscillatory networks in proliferating neural stem cells are constructed in a remarkably similar manner [68, 69]. In myogenic and neural cells, oscillatory HES1 suppresses *MyoD* and *Ascl1*, respectively. Both MYOD and ASCL1 oscillate, and as a consequence of this rhythmic repression the cells remain in an undifferentiated state. Prior to entry into terminal myogenic and neuronal differentiation, MYOD and ASCL1 expression becomes sustained.

Whether other factors of the HES/HEY family participate in the regulation of the oscillatory network has not been investigated. In the nervous system, *Hes/Hey* genes function redundantly as shown by genetic analyses in mice, i.e. loss of one gene of the family can be compensated by other genes of the family [70]. However, cooperative functions of HES/HEY proteins also have to be considered. Homo- or heterodimers of HES/HEY factors have distinct affinities to target sequences, and heterodimers can bind with higher affinities to DNA than homodimers in MuSC and other cell types [28, 71]. Thus, several factors of the *Hes/Hey* family might cooperate in order to control the oscillatory network.

Modeling of the oscillatory network

Mathematical modeling can be used to describe the oscillatory network that was experimentally described in muscle progenitors and MuSC of mice. A first ordinary differential equation model qualitatively predicted the network dynamics in a single cell, and relied on previously published parameters for *Dll1*, *Hes1*, and *MyoD* mRNA and protein stability [68, 72-74]. The model predicted that all three proteins HES1, MYOD, and DLL1 oscillate with identical periods, and was thus in accordance with experimental observations (Fig. 1D). It also correctly predicted the effect of *Hes1* ablation on MYOD and DLL1 oscillations, and the effect of a *MyoD* ablation on DLL1 oscillations [38]. It should be noted that due to differences in stability and in the time

needed for transcription/translation (see also below), phase shifts between the HES1, MYOD and DLL1 oscillations occur (Fig. 1D).

An extension of the mathematical framework made it possible to simulate the expression dynamics in coupled cells, relying on parameters used in single cells and on additional experiments that had defined additional aspects of Notch signal transduction [68, 75]. The coupled-cell delay differential equation model predicted that in two coupled cells, DLL1 will oscillate in both cells, and that these oscillations occur with a shift of half a period, which was subsequently experimentally verified (Fig. 2A). The model therefore can accurately describe oscillatory dynamics of the coupled system, as well as the consequences if one component of the system is removed.

The model can be used to identify strategies for altering oscillations. The delay time τ21 used in the model (Fig. 2A) reflects the time that HES1 protein needs to affect DLL1 protein levels. This delay includes the time needed for *Dll1* transcription, processing and translation of the *Dll1* transcript. Among these complex steps, the *Dll1* transcription time can be manipulated experimentally by changing the length of the primary transcript. This was achieved by the construction of a mutant allele that fused *Dll1-luciferase* cDNA behind the translational start codon of *Dll1*, but left the remainder of the *Dll1* gene intact (*Dll1*^{type2} mutation, see Fig. 2B and [68]). Compared to the *Dll1*^{luc} reporter allele that served as a control in such experiments, the additional sequences in the *Dll1*^{type2} allele increased the overall time needed for the transcription of the *Dll1* locus by approximately 0.1 hour [68]. The model predicted that in a single cell, this increase would neither affect the oscillatory expression nor period. However, in coupled cells, the increased delay is predicted to severely quench the entire oscillatory system (Fig. 2C; and [38]).

Oscillations in cell communities versus cell autonomous oscillations

Before assessing the impact of the *Dll1*^{type2} mutation on the oscillatory network, a series of control experiments were performed in which MuSC that carried either the *Dll1*^{luc} reporter or the *Dll1*^{type2} mutation were compared (see Fig. 2B for a comparison of the *Dll1*^{luc} and *Dll1*^{type2} alleles). Please note that from these two alleles, identical

proteins are produced despite the fact that the transcripts encoding them are distinct. The experimental analysis demonstrated that similar levels of *Dll1* mRNA were present in *Dll1^{luc}* and *Dll1^{type2}* cells, and that Notch target genes (*Hes1, Hey1* and *Hes5*) in contacting MuSC were induced to similar levels [38].

Cells derived from mice carrying the *Dll1^{type2}* allele were used to visualize the consequences of increasing *Dll1* transcription time on the expression dynamics in muscle progenitors and activated MuSC. As predicted by the mathematical model, in single isolated cells the mutation did not change the DLL1 oscillatory behavior. However, whenever cells were contacting each other DLL1 oscillations were severely quenched, resulting in sustained DLL1 expression. Sustained expression was observed in communities of muscle progenitors in cultured slices from limbs of developing mice, as well as in myospheres generated form adult MuSC, or in activated MuSC cells on single myofibers where the cells form colonies [38].

Sustained DLL1 expression in *Dll1^{type2}* mutants had severe functional consequences, resulting in premature differentiation and impairing self-renewal of MuSC. These functional deficits were apparent in muscle progenitors and MuSC in culture and *in vivo*, despite the fact that the *Dll1^{type2}* mutation did not alter overall expression levels of *Dll1* nor the expression of Notch target genes. The *Dll1^{luc}* allele served again as a control for phenotyping experiments; of note, *Dll1^{luc}* neither affects muscle formation nor the number of MuSC in the adult [38]. Comparison of the severity of the phenotypes of *Dll1* null and *Dll1^{type2}* mutations showed that the null mutation affects muscle formation and regeneration more severely. Thus, oscillating DLL1 suppresses myogenic differentiation more effectively than sustained DLL1, and sustained DLL1 produced in *Dll1^{type2}* mutants retains partial functionality [38]. These observations show that in communities of muscle progenitors and MuSC, DLL1 oscillations drive the oscillatory network. Note that the *Dll1^{type2}* mutation also enhanced differentiation of neuronal stem cells, demonstrating a conserved role of DLL1 oscillation in myogenesis and neurogenesis [68]. In summary, in communities of cells that receive and provide Notch signals, the appropriate timing of the DLL1 signal is important to stabilize the oscillatory system, and its oscillatory expression is needed for achieving the correct balance between self-renewal and differentiation.

Two roles of Notch - self-renewal and quiescence.

MuSC numbers decline during the postnatal phase when they also become quiescent. The first Pax7+ cells that exited the cell cycle are observed at birth, and their proportion increases over the next 1-2 months, until in mature mice the vast majority of MuSC are quiescent [76, 77]. The Notch signaling pathway orchestrates quiescence as well as self-renewal in MuSC. It is possible that distinct ligands and/or ligand sources act on the stem cells to control these distinct processes. Cell-type specific ablation in MuSC had indicated that oscillatory DLL1 produced by activated stem cells controls self-renewal of neighboring stem cells. However, in quiescence, MuSC are not contacted by other MuSC. Thus, other ligand sources control quiescence, and recent data indicate that not DLL1 but rather DLL4 controls this.

Myofibers directly contact MuSC and express *Dll4* [14, 16, 78]. In addition, endothelia that express *Dll4* were proposed to contact MuSC in their niche, and several lines of evidence indicate that the ligand that controls quiescence of MuSC corresponds to DLL4 [14, 78-80]. In this context, it is interesting to note that DLL1 and DLL4 are functionally non-equivalent, and cell culture experiments using synthetic biological Notch networks indicate that DLL1 and DLL4 elicit pulsed and sustained responses in signal-receiving cells, respectively [81, 82].

That myofibers provide a Notch signal that keeps MuSC in quiescence is in keeping with experiments where *Mindbomb-1* was ablated in muscle fibers, which interfered with MuSC entry into quiescence [79]. *Mindbomb-1* encodes a RING ubiquitin ligase that is essential for Notch signaling, and interacts with the intracellular domain of Notch ligands to promote their ubiquitylation and internalization. It thus functions in the signal-sending cell, but it is needed for efficient activation of the receptor in the neighboring signal-receiving cell. *Mindbomb-1* is expressed in the myofiber, and its expression is upregulated during puberty when the majority of MuSC exit the cell cycle [79]. Thus, enhanced *Mindbomb-1* expression was proposed to enhance the signaling capacity of the fiber-derived ligand DLL4 in puberty and drive the MuSC out of the cell cycle [14, 79].

Outlook

Regulatory molecules that control stem cell fate can oscillate and/or fluctuate stochastically [46, 68, 69, 83-85]. Dynamics of expression of regulatory factors can encode distinct information and result in different biological outcomes. For instance, oscillatory or sustained ASCL1 expression determines whether neural progenitors will retain their proliferative capacity or differentiate, and oscillatory or sustained signaling of p53 in response to stress controls distinct outcomes like cell cycle arrest or apoptosis [46, 69, 86]. Similarly, the well-studied yeast transcription factor Msn2 regulates multi-stress responses and exhibits oscillatory translocations to the nucleus [87, 88]. Again, distinct Msn2-responsive genes are activated by different Msn2 expression dynamics [89]. Thus, expression dynamics encodes information in various cell types but key questions about expression dynamics and the decoding mechanisms remain unresolved [66]. Theoretical work identified regulatory circuits as candidates that might decipher dynamics [90]. Furthermore, mechanisms of dynamic decoding by target promoters have been examined in yeast [89, 91]. Nevertheless, in most contexts the mechanisms that decode expression dynamics remain little understood.

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Declaration of competing interests

We have no conflicting interests.

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Figure Legends

Figure 1. The oscillatory network comprising HES1, MYOD and DLL1

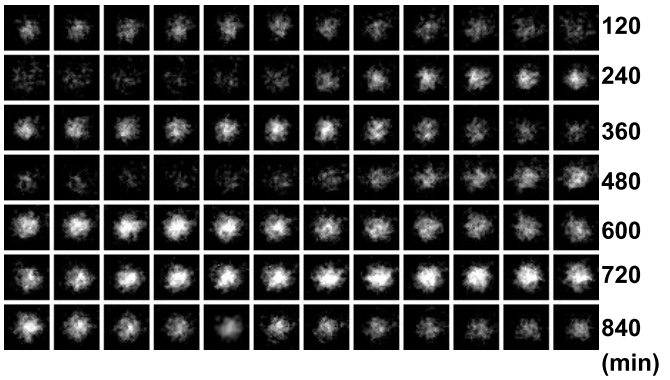
(A) Left: Bioluminescence images of the dynamic expression of luciferase produced from a *Dll1-luciferase* fusion allele. The bioluminescence signals were observed in a single muscle stem cell associated with a cultured myofiber (‘floating fiber; see also [38]); imaging started after fibers were incubated overnight. **Right:** Quantification of the bioluminescence signal shown on the left. **(B) Left:** Bioluminescence images of the dynamic expression of luciferase produced from a *Dll1-luciferase* fusion allele in a muscle stem cell in which *Hes1* was ablated; the cell was associated with a cultured myofiber. **Right:** Quantification of the bioluminescence signal shown on the left. **(C)** Scheme of the oscillatory network and of the regulation of its individual components. HES1 protein (red) represses the transcription of *Hes1*, *Dll1* and *MyoD* (all shown in black*)*, MYOD protein (blue) positively regulates the transcription of *Dll1* (black). DLL1 protein is indicated in green. **(D)** Expression dynamics of DLL1, MYOD and HES1 proteins in a single cell predicted by the ordinary differential equation model.

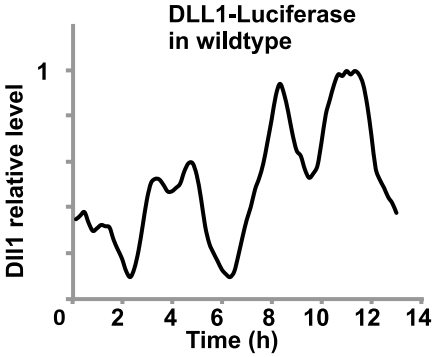
Figure 2. Stable oscillations in coupled cells depend on an appropriate timing of the expression of network components

(A) Left: Scheme of the DLL1 and HES1 regulatory mechanism in two coupled wildtype cells underlying the coupled-cell delay differential equation model. In each cell, HES1 represses its own as well as DLL1 transcription in a cell-autonomous manner. In addition, DLL1 in one cell induces HES1 in the neighboring cell. $\tau 1$ represents the time the HES1 protein needs to regulate its own protein production, $\tau 21$ represent the time that HES1 requires to affect DLL1 protein, and $\tau 22$ is the time that DLL1 in cell1 requires to affect HES1 levels in the neighboring cell 2. **Right:** Simulation of the dynamic expression of DLL1 in two coupled wildtype cells. See [38] for detailed information about the derivation and parametrization of the mathematical models. The model accurately predicts the experimentally observed oscillations in wildtype cells that contact each other [38]. **(B)** Schematic display of the *Dll1* wildtype

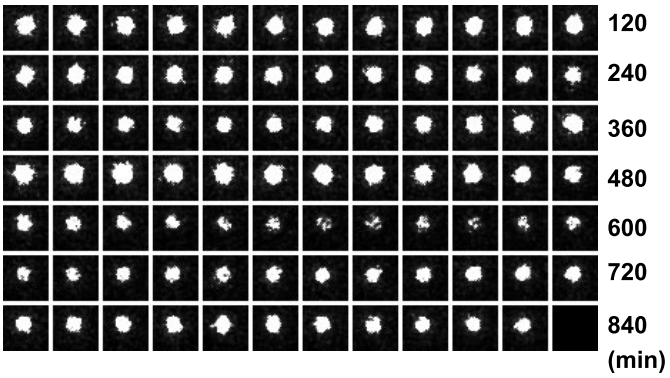
allele, the *Dll1*^{luc} reporter allele and the *Dll1*^{type2} mutant allele. It should be noted that *Dll1*^{luc}/*Dll1*^{luc} mice do not display changes in muscle formation or MuSC numbers, in contrast to the *Dll1*^{type2} mutants show deficits in muscle formation and repair. **(C) Left:** Scheme of the DLL1 and HES1 regulatory mechanisms in two coupled cells carrying the *Dll1*^{type2} mutation. Note that compared to wildtype cells, τ21 is increased by 0.1h due to the increased time required for transcription from the *Dll1*^{type2} mutant allele. **Right:** Simulation of the dynamic expression of DLL1 in two coupled *Dll1*^{type2} mutant cells. The model accurately predicts the experimentally observed oscillations in *Dll1*^{type2} mutant cells that contact each other [38].

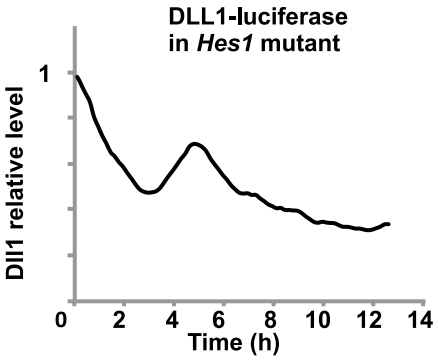
A DLL1-Luciferase expression in wildtype



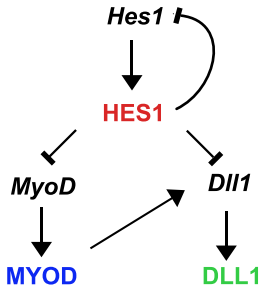


B DLL1-Luciferase expression in *Hes1* mutant

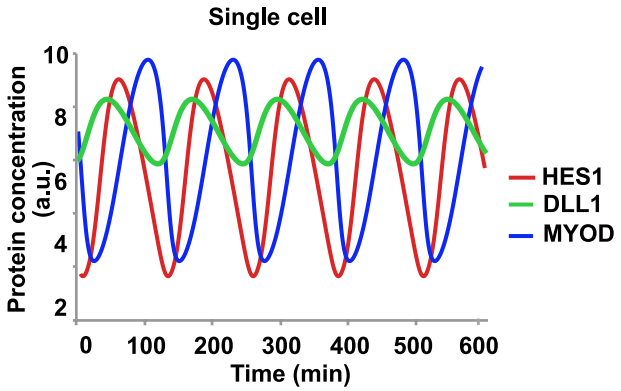


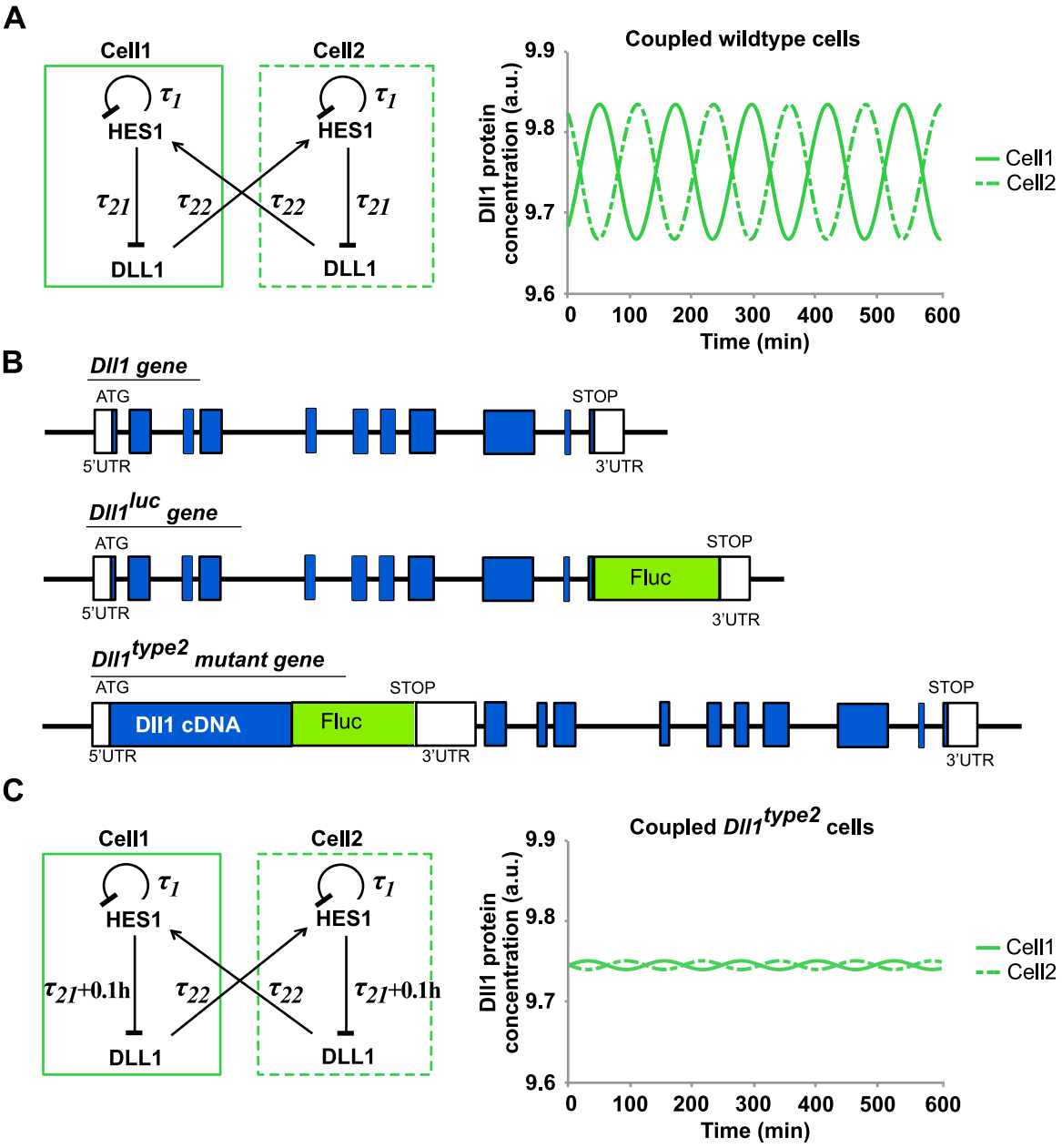


C



D




A
Cell1
Cell2
τ1
HES1
τ21
τ22
DLL1
Coupled wildtype cells
Dll1 protein concentration (a.u.)
9.9
9.8
9.7
9.6
0
100
200
300
400
500
600
Time (min)
Cell1
Cell2
B
Dll1 gene
ATG
STOP
5'UTR
3'UTR
Dll1luc gene
Fluc
Dll1type2 mutant gene
Dll1 cDNA
C
τ21+0.1h
Coupled Dll1type2 cells