Acquisition and extinction of gene expression programs are separable events in heterokaryon reprogramming

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Summary

Although differentiated cells normally retain cell-type-specific gene expression patterns throughout their lifetime, cell identity can sometimes be modified or reversed in vivo by transdifferentiation, or experimentally through cell fusion or by nuclear transfer. To examine the epigenetic changes that are required for the dominant conversion of lymphocytes to muscle, we generated heterokaryons between human B lymphocytes and mouse C2C12 myotubes. We show that within 2 days of heterokaryon formation lymphocyte nuclei adopt an architecture resembling that of muscle and then initiate the expression of muscle-specific genes in the same temporal order as developing muscle. The establishment of this muscle-specific program is coordinated with the shutdown of several lymphocyte-associated genes. Interestingly, erasing lymphocyte identity in reprogrammed cells requires histone deacetylase (HDAC) activity. Inhibition of HDAC activity during reprogramming selectively blocks the silencing of lymphocyte-specific genes but does not prevent the establishment of muscle-specific gene expression. Successful reprogramming is therefore shown to be a multi-step process in which the acquisition and extinction of lineage-specific gene programs are separable events.

Introduction

The theory of nuclear equivalence – that specialised cells of metazoans possess a gene pool identical to that of the zygote nucleus – has been experimentally examined and debated for over a century (Spemann, 1938; Weismann, 1982). Demonstrations of somatic cell reprogramming, particularly through nuclear transfer and in heterokaryons, have shown that many types of differentiated cells retain flexible lineage potential (Baron and Maniatis, 1986; Blau et al., 1983; Gurdon, 1962; Hochedlinger and Jaenisch, 2002; Wilmut et al., 1997; reviewed by DiBerardino, 1988; reviewed by Surani, 2001). In addition, the constitutive expression of specific transcription factors by certain cell types can sometimes modify or override lineage outcome. For example, expression of individual muscle regulatory factors (MRF) of the MyoD family has been shown to convert a range of non-muscle cell types into muscle (Aurade et al., 1994; Choi et al., 1990; Davis et al., 1987; Weintraub et al., 1989). Similarly, high levels of the transcription factors C/EBPα and C/EBPβ can reprogramme committed mature B-lymphocytes to become macrophages (Xie et al., 2004).

In parallel with these demonstrations of dominant conversion, other studies have revealed that additional factors are necessary to maintain cell identity. These include trithorax and polycomb group proteins, required to retain early developmental decisions (reviewed by Orlando, 2003), and other chromatin modifiers such as MEP-1 and Mi-2 that are essential for maintaining soma-germline distinctions (Unhavaithaya et al., 2002). Collectively, these studies have suggested that differentiated cells require continuous and active regulation to maintain their identity (Blau and Baltimore, 1991). Whereas cell-type-specific gene expression programs are established through a network of transcriptional activators and repressors, epigenetic factors might also be required to maintain specification, perhaps by stabilising chromatin domains (Fisher, 2002; Orlando, 2003; Shin and Mello, 2003). Chromatin remodelling factors, including histone deacetylases (HDAC), are required for ‘resetting’ gene expression, for example by overriding germline-specific states in C. elegans (Shin and Mello, 2003), experimental reprogramming through nuclear transfer or using Xenopus egg extracts (Hansis et al., 2004; Kikyo et al., 2000; Rideout et al., 2001), and in the normal development of mammalian oocytes (Arney et al., 2002; Santos et al., 2002).

Later in ontogeny, cellular differentiation is characterised by the coordinated expression and extinction of genes at specific times. In developing lymphocytes, the regulated expression and silencing of the terminal deoxynucleotidyl transferase (TdT) locus, Dntt, provides a particularly well-studied example (Ernst et al., 1999). Dntt is expressed by immature lymphocytes but
is transcriptionally silenced in response to signalling through the T- or B-cell receptor. A temporal analysis of the chromatin modifications that accompany stable silencing showed that silencing is nucleated at the promoter by the ordered deacetylation of histone H3 Lys9, loss of methylation at H3 Lys4 and methylation at H3 Lys9. This was followed by repositioning of the Dnmt locus to pericentric heterochromatin (Brown et al., 1999) and the bi-directional spreading of repressive histone modifications (Su et al., 2004). The importance of histone deacetylation for initiating the assembly of silent chromatin is underscored by a requirement for Ikaro, a DNA binding factor that is essential for lymphocyte development, that interacts with the HDAC-containing nucleosome remodelling NuRD complex (Koipally et al., 1999) and is required for the appropriate stage-specific silencing of many lymphocyte-associated genes (Sabbattini et al., 2001; Su et al., 2004).

In an experimental context, lymphocytes can be successfully reprogrammed to express early developmental markers such as Oct4 when injected into Xenopus oocytes (Byrne et al., 2003) and occasionally upon transfer into mouse oocytes (Hochedlinger and Jaenisch, 2002; Rideout et al., 2001). Dominant reprogramming of lymphocytes also occurs in cell hybrids generated with embryonic stem cells or germ cells (Tada et al., 1997; Tada et al., 2001). Despite this, we do not yet understand how genome function is epigenetically reset during reprogramming. In this study, we examined the dominant conversion of human lymphocytes to muscle in experimental heterokaryons and investigated the role of HDAC activity in this process. We show that heterokaryon formation results in a rapid increase in nuclear volume and the redistribution of constitutive heterochromatin within lymphocyte-derived nuclei. This is followed over 2-8 days by the de novo expression of human muscle genes in a temporal order that accurately recapitulates gene expression in normal developing muscle. Activation of muscle-specific genes in heterokaryons was associated with declining expression of several lymphocyte-specific genes including CD45, PAX5, CD20 and CD37. Treatment with HDAC inhibitors selectively blocked lymphocyte gene extinction, a result that suggests that, although gene activation and silencing are coordinated events in reprogramming, they are mechanistically distinct.

**Results**

**Increased nuclear volume and redistribution of constitutive heterochromatin are early events in the reprogramming of lymphocytes to muscle**

The potential of human B lymphocytes to convert to muscle was examined by generating stable heterokaryons by using polyethylene glycol (PEG)-mediated fusion with mouse C2C12 myoblasts (Blau et al., 1983; Chiu and Blau, 1984), as illustrated in Fig. 1A. Human and mouse nuclei within the resulting myotubes were distinguished by fluorescent in situ hybridisation (FISH) using differentially labelled probes that selectively recognise human γ-satellite or mouse α-satellite DNA. α-satellite DNA (green) was present in the nuclei of human B cells before (Fig. 1B) and after fusion with mouse myotubes (Fig. 1C) and allowed them to be readily discerned from mouse C2C12 nuclei (labelled red with γ-satellite probe). Human lymphocyte-derived nuclei were also identified by counter-staining with DAPI (Fig. 1C right panel, arrow), which highlights AT-rich regions that surround mouse centromeres and generates punctuate labelling that is selective for mouse nuclei. Reprogramming efficiency in interspecies heterokaryons was judged by expression of the human muscle marker NCAM, detected using a monoclonal antibody 5.1H11 antibody, red). One mouse (DAPI intense foci) and one human (arrows) nuclei are seen. Bars, 10 μm. (E) Expression-kinetics of human NCAM (detected by 5.1H11 antibody) by lymphocyte-derived nuclei (mean ± s.d., n=3, 50 nuclei per experiment).

**Fig. 1.** Reprogramming human B-lymphocytes in mouse C2C12 heterokaryons. (A) Protocol used to generate interspecies (hB×C2) heterokaryons. Mouse C2C12 myoblasts were differentiated into myotubes and fused with human B lymphocytes (hB). Mouse and human nuclei were distinguished by FISH with probes specific for mouse γ-satellite DNA (red) or human α-satellite DNA (green), or by DAPI staining (blue). (B) Confocal image of hB nuclei before fusion. (C) Confocal images of a myotube containing a human (arrow) and a mouse nucleus. (D) Confocal section of a reprogrammed heterokaryon identified by hNCAM expression (5.1H11 antibody, red). One mouse (DAPI intense foci) and one human (arrows) nuclei are seen. Bars, 10 μm. (E) Expression-kinetics of human NCAM (detected by 5.1H11 antibody) by lymphocyte-derived nuclei (mean ± s.d., n=3, 50 nuclei per experiment).
mesoderm-derived cells by C2C12 myotubes (Blau et al., 1985).

Dramatic and rapid changes in the size and architecture of human lymphocyte-derived nuclei were observed upon heterokaryon formation (Fig. 2). The volume of human B cell nuclei increased from approximately 600 μm³ prior to fusion to 1500 μm³ and 1800 μm³ 2 and 4 days after heterokaryon formation, respectively (Fig. 2A,B). The distribution of constitutive heterochromatin within these nuclei was also altered. Prior to fusion, human centromeres were clustered to form five to eight chromocentres per nucleus detected by Calcinosis, Raynaud’s, Esophagus, Sclerodactyly and Telangiectasia antisera (CREST) labelling (Fig. 2C, green) (Alcobia et al., 2000; Brown et al., 2001; Weierich et al., 2003). Two days after heterokaryon formation, CREST signals were more compact, consistent with a reduced number of spatially discrete signals (supplementary material Fig. S1), and the number of chromocentres per nucleus was markedly reduced (Fig. 2C,D). This reorganisation mimicked the spatial distribution of constitutive heterochromatin seen in neighbouring C2C12 nuclei (supplementary material Fig. S1). Changes in nuclear size and architecture were evident before the expression of hNCAM, suggesting that nuclear reorganisation precedes human muscle gene expression (Fig. 2D). The rapid timing of the response implicates transacting factors derived from mouse myotubes in the nuclear remodelling of lymphocytes. Consistent with this possibility, myogenin protein was detected in many human nuclei as early as 24 hours after heterokaryon formation (Fig. 2E), several days before endogenous human myogenin transcripts were expressed (Fig. 3A).

Reprogramming initiates a temporally ordered activation of human myogenic regulatory factors by lymphocyte-derived nuclei in C2C12 heterokaryons

To determine the kinetics of gene activation and silencing induced by heterokaryon formation and reprogramming of human lymphocytes, we analysed the expression of a panel of human muscle- and B cell-associated genes using reverse transcriptase (RT)-PCR and primer combinations that were specific for human transcripts. This showed that muscle gene expression was efficiently initiated in human B cell nuclei 2 days after heterokaryon formation (Fig. 3A). Interestingly, human myogenic regulatory factor (MRF) genes were expressed in a sequence that accurately reflects their normal temporal order (Pownall et al., 2002), so that hMYF5 was transiently expressed at days 2-3, followed by hMYOG (day 3 onwards) and hMRF4 later (day 4 onwards). The establishment of this new gene program by human B-cell-derived nuclei was accompanied by declining expression of the B-cell-specific regulator hPAX5 and the leukocyte-associated gene hCD45. Expression of human GAPDH, a ubiquitously expressed gene, remained constant throughout these analyses.

HDAC activity is required for the silencing of lineage inappropriate genes during reprogramming

To assess the importance of HDAC activity in the dominant reprogramming of lymphocytes to muscle, we treated

Fig. 2. Nuclear reorganisation is an early event in lymphocyte reprogramming. (A) Single optical section showing lamin A/C immunofluorescence-labelling of human B lymphocyte nuclei (hB) before day 0 (d0) and three days (d3) after fusion with C2C12 myotubes. (B) Volume of hB nuclei before (d0), and 2 and 4 days after heterokaryon formation (d2 and d4, respectively) compared with C2C12 myotubes. (C) Distribution of constitutive heterochromatin in hB nuclei before (d0, open bars) and 2 and 4 days after heterokaryon formation (d2 and d4, respectively) compared with C2C12 myotubes 2 and 4 days after serum withdrawal and differentiation (d2 and d4, respectively). Nuclear volume was estimated as described in Materials and Methods (mean ± s.d., n=10 with 50 nuclei per experiment). (C) CREST antisera (green) reveal the centromere distribution in hB nuclei (d0) and two days after heterokaryon formation (d2), where individual chromocentres are indicated (arrowed). Confocal images are maximal projection of multiple optical z-sections. (D) Distribution of constitutive heterochromatin in hB nuclei before (d0, open bars) and 2 days after fusion in hNCAM expressing (d2, black bars) and hNCAM-negative (not yet reprogrammed) heterokaryons (grey bars) was compared by assessing the number of discrete CREST signals and number of chromocentres per nucleus (n=100). (E) Myogenin protein (green) in a human nucleus (arrowheads) 1 day after fusion. Images show single optical sections. Bars, 10 μm.
heterokaryons with the HDAC inhibitor TSA. Application of low doses of TSA to differentiating C2C12 or to primary cultures of mouse muscle has been shown to increased histone H3 Lys9 and histone H4 Lys20 trimethylation at pericentric DNA and prevent centromeric clustering (Terranova et al., 2005), suggesting a role for HDACs in the formation of repressive heterochromatin environments during terminal differentiation. To assess their importance during lineage conversion, heterokaryons established between human B lymphocytes and C2C12 cells were treated with low doses of trichostatin A (TSA, 20 nM). This did not affect the success of heterokaryon formation or the proportion of 5.1H11-positive reprogrammed nuclei 7 days after fusion (79±6.7%, compare with 72±8.7% in absence of TSA), although a slightly enhanced induction of human NCAM expression was seen in the presence of TSA (Fig. 3B). A comparison of MYF5, MYOG and MRF4 expression in heterokaryons that were treated with TSA or not (Fig. 3C) confirmed that acquisition of muscle gene expression by human lymphocyte-derived nuclei was not significantly compromised. MYOG expression was detected at days 4 and 7, MRF4 was most abundant at later stages, whereas MYF5 was only detected early after heterokaryon formation (days 2-3). TSA treatment resulted in a slight advance in the timing of hMRF4 expression, a result that is consistent with a global increase in histone acetylation (Fig. 4D) and chromatin accessibility induced by TSA and a previous report that showed precocious expression of mMRF4 in primary mouse-muscle treated with HDAC inhibitors (Terranova et al., 2005). TSA did, however, have a dramatic effect on the temporal extinction of human lymphocyte gene expression in interspecies heterokaryons (Fig. 4A). Whereas expression of hCD45, hPAX5, hCD20 and hCD37 was extinguished (or significantly diminished in the case of hCD45) in heterokaryons by day 7, sustained expression of these genes was evident in TSA-treated heterokaryons (highlighted in green, compare left and right panels, Fig. 4A). Human GAPDH, a gene that is expressed in all human tissues, was detected throughout in both TSA-treated and untreated cultures, and is shown for comparison.

To evaluate further the selectivity of the TSA response, we analysed the expression of other human genes including hPAX6, hREX1, hTERT, hNANOG and hOCT4. Most genes were not significantly upregulated in heterokaryons upon TSA treatment (Fig. 4B). Similarly, TSA-treated mouse myoblasts and mouse myotubes did not inappropriately express most lymphocyte-associated and neural-associated genes (Fig. 4C), although a minority (two of 11) showed slight upregulation. This result is consistent with estimates that approximately 10% of genes are sensitive to HDAC inhibition in myoblasts (Iezzi et al., 2004). The selectivity of this response is also supported by the finding that hPAX5 extinction in heterokaryons was not associated with a decline in the expression of PAX5 upstream positive regulators (hE2A and hEBF) and that hPAX5 (but not hE2A or hEBF), was sensitive to TSA treatment (supplementary material Fig. S2).

Inhibition of HDAC activity results in the coexpression of hCD20 and hNCAM in individual reprogrammed nuclei

The observation that TSA prevents the silencing of lymphocyte-associated genes but allows the acquisition of muscle gene expression predicts that TSA treated heterokaryons would contain nuclei that simultaneously express lymphocyte and muscle-associated genes. To verify this at the level of individual nuclei, RNA-FISH analysis was performed to detect human muscle- (hNCAM-biotin) and lymphocyte- (hCD20-DIG) specific transcripts following TSA
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The specificity of probes for hCD20 and hNCAM transcripts was verified by the detection of two RNAse-sensitive hCD20 signals (green) in human B lymphocyte nuclei and two hNCAM signals (red) exclusively in primary human myoblasts (Fig. 5A and B, respectively). Using these probes, RNA-FISH analysis was used to score nuclei expressing the muscle gene hNCAM alone, the lymphoid gene hCD20 alone, or coexpressing both hNCAM and hCD20 within heterokaryons at day 5 (illustrated in Fig. 5C, values shown in Fig. 5D and supplementary material Table 1). In untreated heterokaryons, most nuclei (75%) in which human RNA signals were found expressed hNCAM and only a minority (32%) expressed hCD20. Nuclei in which both transcripts were detected simultaneously were rare (7%). Addition of TSA or the structurally unrelated HDAC inhibitor valproic acid (VPA) did not affect muscle-specific hNCAM signals (red histograms) but had a dramatic effect on the proportion of lymphocyte-derived nuclei that retained hCD20 expression (green histograms, Fig. 5D). Consequently, and in contrast to untreated cultures, many lymphocyte-derived nuclei within TSA- or VPA-treated heterokaryons coexpressed both hCD20 and hNCAM (28 and 29%, respectively, Fig. 5D). These results confirm the importance of HDAC activity for silencing lymphocyte-specific genes during reprogramming.

Discussion

Previous studies have shown that lymphocytes can be reprogrammed by injection into Xenopus (Byrne et al., 2003) or mouse oocytes (Hochedlinger and Jaenisch, 2002) or by generating cell hybrids with embryonic stem cells or germ cells (Tada et al., 1997; Tada et al., 2001). In this study, we show that the dominant conversion of human lymphocytes to muscle is a multi-step process that includes changes in nuclear size, architecture and gene activity. In muscle heterokaryons, where chromosome replication is precluded, these steps can be distinguished on the basis of their timing and a requirement for HDAC activity. Within the first 48 hours of heterokaryon formation, lymphocyte nuclei increase in size and heterochromatin domains are redistributed to mimic the spatial arrangement of neighbouring mouse myocytes.
Importantly, this reorganisation precedes the activation of endogenous human muscle-specific genes. It is possible that these changes in nuclear architecture are the result of physical constraints or dominant factors within myotubes that impose a muscle-specific organisation. However, because cycling and non-cycling lymphocytes display different constitutive heterochromatin organisation (Brown et al., 1999; Solovei et al., 2004) and cell-cycle withdrawal is a characteristic feature of myogenic differentiation (Ait-Si-Ali et al., 2004; Shen et al., 2003; Walsh and Perlman, 1997), it is also possible that nuclear reorganisation is a consequence of cell-cycle arrest. Previous studies have shown that fusion of nucleated chicken erythrocytes with rat myoblasts results in pronounced nuclear enlargement and chromatin redistribution prior to gene reprogramming (Dupuy-Coin et al., 1976) and similar effects have been reported for mouse lymphocytes injected into Xenopus oocytes (Byrne et al., 2003) and bone marrow cells that form stable heterokaryons with Purkinje neurons (Weimann et al., 2003). More recently, chromatin redistribution in developing muscle was shown to be dependent on the methyl-CpG-binding proteins MeCP2 and MBD2 (Brero et al., 2005). Taken together, these results suggest that myogenic regulators and chromatin remodelling machinery both have important roles in conveying dominant cell-type-specific nuclear organisation during reprogramming.

Our results show that de novo expression of an endogenous muscle gene program by lymphocyte-derived nuclei begins 2 days after heterokaryon formation and coincides with the reduced expression of several lymphoid-associated genes (hCD45, hPAX5, hCD20, hCD37). Extinction of lymphocyte-specific gene expression begins after 2-3 days and continues over a 7-day period with similar kinetics to the reported shutdown of human albumin expression during hepatocyte reprogramming to muscle (Miller et al., 1988). Remarkably, we show that inhibition of HDAC activity by low doses of TSA or VPA prevents the silencing of lineage-inappropriate genes without affecting ongoing conversion to muscle. This shows that the establishment of muscle gene expression and the extinction of lymphocyte identity are distinct components of lineage conversion. Remarkably, by inhibiting HDAC activity in experimental heterokaryons nuclei are seen to coexpress two different lineage-associated gene programs. This underscores the importance of gene silencing for successful reprogramming and suggests that in future it might be possible to preserve certain characteristics of donor cells through lineage conversion, by restricting the availability of HDACs.

**Materials and Methods**

**Cell culture**

C2C12 cells (Blau et al., 1983; Yaffe and Saxel, 1977) were maintained as undifferentiated myoblasts in growth medium (GM); Dulbecco's modified Eagle’s medium (DMEM) supplemented with 20% foetal calf serum (FCS), 4 mM glutamine and 10 μg ml⁻¹ penicillin and streptomycin. Myotube formation was induced by culturing in low-serum medium (DM: DMEM containing 2% horse
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15 minutes once, then n cycles of 94°C for 15 seconds, 60°C for 30 seconds and 72°C for 60 seconds. The sequence of the human-specific primers and the number of cycles used are indicated in supplementary material Table S2. For each reaction PCR amplification, conditions were within the dynamic range (i.e. non-saturating).

PCR products were separated on agarose gels and blotted on Hybond-N nylon membrane (Amersham Pharmacia Biotech) before hybridisation with 32P-labeled probes (Prime-It II kit, Stratagene). Real-time PCR analysis of hPAX5, hERF and hE2A was carried out on a Chromo4™ DNA engine using Opticon Monitor software (MJ Research Inc), running the following program: 95°C for 15 minutes once, then 40 cycles of 94°C for 15 seconds, 60°C for 30 seconds and 72°C for 30 seconds. PCR reactions included 2X Sybr-Green PCR Mastermix (Qiagen), 300 nM primers and 2 μl of template in a 35 μl reaction volume. Each measurement was performed in triplicate and data were normalised according to human GAPDH expression.

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References

Ai R, Natsume Y, Mutaguchi N, Aizawa S, Yoshida K, Takeya M. (2003). Nuclei of adult mammalian somatic cells are directly reprogrammed to oct-4 stem cell gene expression by amphibian oocytes. Cell 114, 317-320.

Alvarez-Buylla, A. R., and Lendahl. (1992). The nuclear matrix mediates the repression of globin gene expression in differentiated cells. Cell 70, 543-553.

Arai Y, Ozawa K, Takeuchi Y, Otsuka T, Nishiyama N, Muramatsu Y, Nishimura J, Inoue S, Saito Y, Hamada T, Terasaki M, Yamashita N, Ozawa T. (2004). The putative silencing complex for the PAX5 repressor in lymphocytes. J. Exp. Med. 201, 633-640.

Arai Y, Igarashi A, Ozawa K, Takeuchi Y, Otsuka T, Nishiyama N, Muramatsu Y, Nishimura J, Inoue S, Saito Y, Hamada T, Terasaki M, Yamashita N, Ozawa T. (2005). The chromatin remodelling complex SHARP forms a putative silencing complex for the PAX5 repressor in lymphocytes. J. Exp. Med. 201, 633-640.

Arai Y, Igarashi A, Ozawa K, Takeuchi Y, Otsuka T, Nishiyama N, Muramatsu Y, Nishimura J, Inoue S, Saito Y, Hamada T, Terasaki M, Yamashita N, Ozawa T. (2006). The chromatin remodelling complex SHARP forms a putative silencing complex for the PAX5 repressor in lymphocytes. J. Exp. Med. 201, 633-640.

Arai Y, Igarashi A, Ozawa K, Takeuchi Y, Otsuka T, Nishiyama N, Muramatsu Y, Nishimura J, Inoue S, Saito Y, Hamada T, Terasaki M, Yamashita N, Ozawa T. (2007). The chromatin remodelling complex SHARP forms a putative silencing complex for the PAX5 repressor in lymphocytes. J. Exp. Med. 201, 633-640.

Arai Y, Igarashi A, Ozawa K, Takeuchi Y, Otsuka T, Nishiyama N, Muramatsu Y, Nishimura J, Inoue S, Saito Y, Hamada T, Terasaki M, Yamashita N, Ozawa T. (2008). The chromatin remodelling complex SHARP forms a putative silencing complex for the PAX5 repressor in lymphocytes. J. Exp. Med. 201, 633-640.
Fisher, A. G. (2002). Cellular identity and lineage choice. Nat. Rev. Immunol. 2, 977-982.

Gurdon, J. B. (1962). Adult frogs derived from the nuclei of single somatic cells. Dev. Biol. 4, 256-273.

Hansis, C., Barreto, G., Maltry, N. and Niehrs, C. (2004). Nuclear reprogramming of human somatic cells by xenopus egg extract requires BRG1. Curr. Biol. 14, 1475-1480.

Hochstedler, K. and Jaenisch, R. (2002). Monoclonal mice generated by nuclear transfer from mature B and T donor cells. Nature 415, 1035-1038.

Iezzi, S., Di Padova, M., Serra, C., Caretti, G., Simone, C., Maklan, E., Minetti, G., Zhao, P., Hoffman, E. P., Puri, P. L. et al. (2004). Deacetylase inhibitors increase muscle cell size by promoting myoblast recruitment and fusion through induction of follistatin. Dev. Cell 6, 673-684.

Kikyo, N., Wade, P. A., Guschin, D., Ge, H. and Wolffe, A. P. (1999). Repression by Ikaros family factors and the specification of muscle progenitors in vertebrate embryos. Genes Dev. 13, 235-246.

Koopally, J., Renold, A., Kim, J. and Georgopoulos, K. (1999). Repression by Ikaros and Aiolos is mediated through histone deacetylase complexes. EMBO J. 18, 3090-3100.

Lawrence, J. B., Taneja, K. and Singer, R. H. (1989). Temporal resolution and sequential expression of muscle-specific genes revealed by in situ hybridization. Dev. Biol. 133, 235-246.

Miller, S. C., Pavlath, G. K., Blakely, B. T. and Blau, H. M. (1988). Muscle cell components dictate hepatocyte gene expression and the distribution of the Golgi apparatus in heterokaryons. Genes Dev. 2, 330-340.

Orlando, V. (2003). Polycomb, epigenomes, and control of cell identity. Cell 112, 599-606.

Pownall, M. E., Gustafsson, M. K. and Emerson, C. P., Jr. (2002). Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu. Rev. Cell Dev. Biol. 18, 747-783.

Rideout, W. M., 3rd, Eggan, K. and Jaenisch, R. (2001). Nucleolar cloning and epigenetic reprogramming of the genome. Science 293, 1093-1098.

Sabbatini, P., Lundgren, M., Georgiou, A., Chow, C., Warnes, G. and Dillon, N. (2003). Polycomb, epigenomes, and control of cell identity. Cell 112, 599-606.

Shen, X., Collier, J. M., Hsuing, M., Zhang, L., Delshad, E. H., Bristow, J. and Bernstein, H. S. (2003). Binding of Ikaros to the lambda5 promoter silences transcription through a mechanism that does not require heterochromatin formation. EMBO J. 20, 2812-2822.

Santos, F., Hendrich, B., Reith, W. and Dean, W. (2002). Dynamic reprogramming of DNA methylation in the early mouse embryo. Dev. Biol. 241, 172-182.

Shin, T. H. and Mello, C. C. (2003). Chromatin regulation during C. elegans germline development. Curr. Opin. Genet. Dev. 13, 455-462.

Solovei, I., Schermelleh, L., During, K., Engelhardt, A., Stein, S., Cremer, C. and Cremer, T. (2004). Differences in centromere positioning of cycling and postmitotic human cell types. Chromosoma 112, 410-423.

Spermann, H. (1938). Embryonic Development and Induction. New York: Hafner Publishing Company.

Su, R. C., Brown, K. E., Saaber, S., Fisher, A. G., Merkenschlager, M. and Smale, S. T. (2004). Dynamic assembly of silent chromatin during thymocyte maturation. Nat. Genet. 36, 502-506.

Surani, M. A. (2001). Reprogramming of genome function through epigenetic inheritance. Nature 414, 122-128.