Endogenous Myc controls mammalian epidermal cell size, hyperproliferation, endoreplication and stem cell amplification

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Summary
The transcription factor Myc (c-Myc) plays an important role in cell growth and cell death, yet its physiological function remains unclear. Ectopic activation of Myc has been recently suggested to regulate cell mass, and Drosophila dmyc controls cellular growth and size independently of cell division. By contrast, it has been proposed that in mammals Myc controls cell division and cell number. To gain insights into this debate we have specifically knocked out Myc in epidermis. Myc epidermal knockout mice are viable and their keratinocytes continue to cycle, but they display severe skin defects. The skin is tight and fragile, tears off in areas of mechanical friction and displays impaired wound healing. Steady-state epidermis is thinner, with loss of the proliferative compartment and premature differentiation. Remarkably, keratinocyte cell size, growth and endoreplication are reduced, and stem cell amplification is compromised. The results provide new and direct evidence for a role for endogenous Myc in cellular growth that is required for hyperproliferative cycles and tissue homeostasis.

Keywords: Cellular growth, Proliferation, Endoreduplication, Cell renewal, Polyploidy

Introduction
The coordination between cell growth and cell division is crucial to embryogenesis, adult tissue homeostasis and carcinogenesis. Recent studies have placed Myc at the centre of this issue. Although a role of Myc in cell cycle progression and proliferation is well established, it has been recently shown that ectopic over-activation of Myc induces increased protein and RNA synthesis and increased cell size (Grandori et al., 2000; Gandarillas et al., 2000; Kim et al., 2000). Accordingly, Myc candidate targets include genes involved not only in cell cycle and apoptosis, but also in cell metabolism and protein and RNA biosynthesis (Levens, 2002; Gomez-Roman et al., 2003). Study of the function of the endogenous gene has been hampered because Myc whole-body knockout (KO) mouse embryos do not survive beyond embryonic day 10 (E10) (Davis et al., 1993). Interestingly, in invertebrates, Drosophila dmyc mutants are smaller and contain smaller cells (Johnston et al., 1999). However, Trumpp et al. (Trumpp et al., 2001) obtained mice with reduced Myc expression by allelic combinations and reported that body and organs were smaller, but there was no apparent decrease in cell size. They suggested that in contrast to dmyc, the mammalian gene is not essential for cellular growth and instead controls the cell decision to divide or not to divide.

To directly investigate the role of endogenous Myc in cell growth control in vivo, we have used the CRE/lox system to specifically inactivate the gene in epidermis, a tissue that requires continuous cell multiplication and enlargement. Ectopic constitutive activation of Myc in human primary keratinocytes or mouse epidermis drives stem cells into clonal expansion, maturation and differentiation (Gandarillas and Watt, 1997; Waikel et al., 2001; Arnold and Watt, 2001). However, the function of the endogenous gene in epidermis remains unknown. Interestingly, ectopic activation of Myc in primary keratinocytes results also in increased cell size and increased endoreplication (cycles of DNA replication in the absence of cell division) (Gandarillas et al., 2000). Endoreplication has been shown to be linked to cellular growth in Drosophila and plants, and may therefore participate in the biological actions of Myc. Here we report endogenous Myc functions in the link between cell growth and cell division in mammalian epidermis.

Materials and Methods
Generation of K5Cre: Myclox +/+ transgenic mice and genotyping
Mice carrying a floxed Myc allele (Myclox ++/) (de Alboran et al., 2001) were bred to transgenic mice expressing the Cre recombinase under the control of the proliferative keratinocyte-specific keratin 5 promote (Ramirez et al., 2004). The genetic background was B6×DBA2 for K5Cre and 129 for Myclox. Genotyping of the animals was performed by PCR on genomic DNA from tail or fingers, using
for 2 hours or overnight (O/N) before they were sacrificed. Paraffin sections were stained with anti-BrdU antibody (Becton Dickinson) followed by indirect immunofluorescence. Either type of experiment positive cells were counted with respect to total basal nuclei after two independent experiments. Sections were stained as described above.

BrdU label-retaining analyses

Ten-day-old pups were injected with 20 µl of 50 µg/g body weight BrdU three times every 12 hours to label DNA replicating cells. Three control and three KO mice of the same litter and gender were sacrificed after the last injection. Five control and five KO mice were sacrificed 2 months 12 hours after the last injection. In either case, positive cells were counted with respect to total basal nuclei after two independent experiments. Sections were stained as described above.

Experimental wound healing

KO and control mice were shaved, mice were anaesthetised and 3 mm skin punch biopsies were made on each side of the back. Wounds were observed until closure or animals were killed, the wound excised and fixed for histology. BrdU incorporation on the healing fronts was performed and analysed as above. The experiment was repeated independently seven times with controls and KO littermates. Mice were aged 2 months to one and half years old.

Oil-Red-O staining

Oil-Red-O (ORO) staining was performed on frozen skin sections from five pairs of control and KO littermates aged from 3 months to
Myc epidermal knockout mice

1 year. Slides were stained in ORO 1% in 60% aq. iso-propyl-alcohol for 15 minutes and in half strength Harris’ hematoxylin.

Primary keratinocyte culture and immunofluorescence
Mouse keratinocytes from back skin were cultured on 12-3T3 feeder layer as described previously (Romero et al., 1999) on collagen IV-coated dishes (Becton Dickinson). For immunofluorescence, coverslips were subsequently fixed in formaldehyde 4% and –20 cold methanol and then immunostained (briefly, 1 hour with primary antibody and 1 hour with fluorescent secondary antibody) and stained with DAPI for DNA before mounting.

Cell migration
Keratinocytes were freshly isolated from control or KO mice. They were plated and cultured in serum free medium (SFM, Invitrogen) supplemented with EGF (epidermal growth factor, 5 ng/ml) and BPE (bovine pituitary extract, 50 µg/ml). Keratinocytes were time-lapse recorded for 15 hours. Pictures were taken every 4 minutes. Migration was quantitated using Metamorph Trackpoint function taking into account elongated-moving cells. The experiment was done with two pairs of control and KO littersmates aged 1 or 6 months; 4 fields and 40 tracks were established per animal and treatment.

Results
Ablation of Myc in epidermis
Knockin mice in which the wild-type Myc gene has been substituted for the same gene flanked by loxP sequences (deAlboran et al., 2001) were crossed with mice expressing CRE under a keratin K5 promoter. This promoter is active in proliferative, basal keratinocytes of stratified epithelia (such as epidermis, oral epithelium, tongue, oesophagus, part of the stomach) from day 14 of development onwards (Ramirez et al., 1994; Ramirez et al., 2004). Recombination of the loxP sequences flanking exons 2 and 3 of the Myc gene suppresses the mRNA and protein expression (deAlboran et al., 2001). We confirmed the keratinocyte-specificity of CRE activity by mice bearing a GFP-lox marker that is expressed upon CRE action (Mao et al., 2001). Within skin, GFP is only expressed in keratinocytes expressed upon CRE action (Mao et al., 2001). For immunofluorescence, coverslips were subsequently fixed in formaldehyde 4% and –20 cold methanol and then immunostained (briefly, 1 hour with primary antibody and 1 hour with fluorescent secondary antibody) and stained with DAPI for DNA before mounting.

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RNase protection assays (RPAs) showed the loss of Myc messenger in KO epidermis (Fig. 1D). We found a slight upregulation of the MYC family member L-myc in young mice not displaying a severe phenotype. N-myc was very hardly detected (the strongest case is shown in Fig. 1D) and B-myc was markedly downregulated in the absence of Myc. Both N-myc and L-myc have been found able to replace Myc in some contexts (Resar et al., 1993; Morgenbesser et al., 1995; Yin et al., 1999; Landay et al., 2000; Malynn et al., 2000). Therefore, some possible compensatory effect by L-myc in younger mice cannot be ruled out. L-myc was nevertheless downregulated in older epidermal KO mice presenting a severe phenotype (Fig. 1D). The messenger of Myc inhibitor mad1 was consistently upregulated.

Tight and fragile skin
The skin of epidermal Myc KO newborns was tighter and shinier (Fig. 2A). Adult KO mice had difficulty extending body limbs and displayed a more compacted appearance (Fig. 2B). Newborns and adult KO animals were smaller, although no signs of underfeeding were observed (Fig. 2A,B and not shown). Their skin was tighter and more fragile, and the mice developed a severe phenotype at around 3 or 4 months of age when the skin had begun to develop local tearing (Fig. 2C-E). This occurred mainly in the face, lips, neck, abdomen and under the arms – areas of mechanical tension and friction. The
spontaneous skin gaps had difficulty healing and in some cases the animal had to be sacrificed. When the skin managed to close up it was tighter and the animal had even more difficulty extending limbs (Fig. 2F,G). Hair loss was often observed in mice with a severe phenotype, and in rare cases body hair was completely lost (Fig. 2G). In these cases spontaneous lesions were detected all over the body. Ct, control littermates with neither Cre nor Myclox alleles (wt), with just Cre, or with just myclox.

Epidermal keratinocytes continue to cycle but differentiate prematurely

Myc KO epidermis was thinner than controls and presented signs of atrophy (Fig. 3). There were gaps in the line of basal nuclei indicating loss of cellularity in the proliferative layer (Fig. 3A,B; arrowheads), and basal nuclei were smaller and often flattened as normal suprabasal nuclei (compare Ct and KO nuclei in all Fig. 3, especially with respect to normal dermal nuclei in more magnified images of K1/BrdU; Fig. 3D). There was also some loss of the proliferative marker keratin K5 (Fig. 3C) and in some areas mild hyperkeratosis (excess of squamous layers; e.g. arrowhead Fig. 3B,C). Markers of early terminal differentiation, K1 and K10, were extensively expressed in the basal layer of KO epidermis, where later differentiation markers involucrin and filaggrin were also often detected (Fig. 3C). The epidermal basal layer is normally proliferative. Altogether this indicates that differentiation initiates prematurely in the absence of Myc, possibly because of a proliferation defect. However, in situ cell cycle analyses provided somewhat surprising results. The proportion of DNA replicating cells (in vivo BrdU incorporation) in KO epidermis was only around 20% reduced when compared with controls (Fig. 3D,E), and the proportion of cells expressing Ki67, a marker of active cell cycle, was not significantly different (Fig. 3D-F). This was also the case in mice that had a severe phenotype and lower L-myc mRNA expression (Fig. 3D). Therefore, keratinocytes continued to cycle in the absence of Myc and their proliferative capacity seemed to be only slightly decreased in steady-state epidermis. However, the size of epidermis was reduced and there was loss of cellularity in the proliferative layer, suggesting a cell growth defect.

Endogenous Myc controls cell size and endoreplication

We wished to study whether the reduced proliferative compartment in the absence of Myc might be, at least in part, due to differences in cellular growth. We therefore analysed cell size and cell cycle (DNA content) of freshly isolated keratinocytes from control or Myc KO epidermis by flow cytometry. As keratinocytes differentiate they increase in cell size (Banks-Schlegel and Green, 1981). Human basal or differentiating keratinocytes can be identified on the basis of their light scatter parameters (Jones and Watt, 1993; Gandarillas et al., 2000), and some evidence suggests this is also the case for mouse keratinocytes (Romero et al., 1999). To determine the distribution of mouse proliferating and differentiating cells in the light scatter plot we stained for the antiproliferative, terminal differentiation markers K1 or K10, and involucrin (Fig. 4B, and not shown). Differentiating and non-differentiating cells of control mice showed a similar light scatter distribution to human keratinocytes. KO epidermis contained a smaller proliferative compartment relative to the differentiating population and compared with control epidermis (Fig. 4A,B). KO epidermis was more differentiated on a morphological basis (light scatter) and on the basis of expression of differentiation markers (Fig. 4A,B). Interestingly, KO keratinocytes were strikingly smaller (forward scatter) than controls, and this was especially remarkable in differentiating cells (Fig. 4A,C).

To determine the effect of Myc loss on the cell cycle distribution, we stained epidermal keratinocytes for DNA content (Fig. 4D,E). KO basal keratinocytes had accumulated slightly more in G1 than in control cells, but overall their DNA profile was not importantly changed. More substantial effects were found in the cell cycle of KO differentiating cells. A significant proportion of suprabasal cells from control mice had a 4N DNA content or were polyploid, as we have observed in human keratinocytes and epidermis (Fig. 4D) (Gandarillas et al., 2000) (J.Z. et al., unpublished). By contrast, the G1 peak
was augmented and the 4N and polyploid populations were significantly reduced in the Myc KO epidermis. Interestingly, no polyploid cells were found in the K1 or K10 negative, proliferative population, whereas half of K1 or K10 expressing cells were polyploid in control mice (Fig. 4E and not shown). By contrast, only a fourth of terminally differentiating cells were polyploid in the KO epidermis. The size of keratinocytes correlates with their DNA content (Gandarillas et al., 2000) (Fig. 4F) and this was also the case in Myc KO epidermis. However, KO keratinocytes were smaller than controls for every phase of the cell cycle, especially in the case of suprabasal, differentiating cells (Fig. 4F and not shown). Thus, the effect of Myc on keratinocyte cell size was at least partly independent of the cell cycle.

Skin regeneration, keratinocyte hyperproliferation and stem cell function are compromised

The long-healing spontaneous lesions and the cell growth defects suggested that epidermal renewal and skin regeneration might be impaired. To test and monitor this we performed experimental wound-healing. Punch wounds of 3 mm took longer to heal in the KO mice than in the control littermates (Fig. 5A-H). Histological analyses revealed a poor healing front in epidermal KOs, with fewer layers of keratinocytes (compare Fig. 5D,H). Interestingly, in this case cell cycle was importantly reduced as revealed by a consistent marked difference in BrdU incorporation (Fig. 5J,N,K). β1 integrin, an epidermal hyperproliferative marker, was also markedly reduced in the KO healing front (Fig. 5I,M). Keratin K6 that is expressed in wild-type healing epidermis was also detected in the thinner healing front of the KO mice (not shown). The hyperproliferation that accompanies wound healing was therefore significantly impaired in the absence of Myc. To explore whether the healing defect was mainly due to the proliferation defect or also to defective migration, we analysed by time-lapse filming elongated cell motility that might be involved in wounding two-dimensional cell migration (Etienne-Manneville and Hall, 2001). Migration of KO keratinocytes was not impaired, rather slightly stimulated when
Fig. 4. Cell size and cell cycle analyses. (A-F) Representative flow cytometry analyses of keratinocytes isolated from control (Ct) or KO epidermis of skin as indicated. (A) Light scatter dot plot of whole populations. R1, region gating smaller, blunt, basal cells; R2, region gating larger and more complex, suprabasal (suprab) cells. (B) Keratin 1 (K1; FL-1) expression of cells in R2 or R1; numbers in brackets are percent of K1-negative cells (left histograms) or K1-positive cells (right histograms) within the total population; K1-positive cells are in green and K1-negative cells in red in the light scatter dot plots. (C) Overlays of cell size (forward scatter, FSC) of the populations indicated. (D) DNA content (FL2) profiles of basal (R1) or suprabasal (R2) keratinocytes. (E) DNA content profiles of proliferative (K1−) or differentiating (K1+) cells. M2 for the G1 phase; M1 for polyploid cells. (F) Cell size (FSC) versus DNA content (FL2); a line has been drawn to help comparing FSC values. 10,000 keratinocytes were counted for every sample (see Materials and Methods). Numbers are percent of cells in each gate within each plot.
Myc epidermal knockout mice compared with controls (Fig. 5O,L,P) and in agreement with inhibited migration of keratinocytes overexpressing Myc (Waikel et al., 2001; Frye et al., 2003). This suggests that defective wound-healing KO epidermis was mainly due to defects in cell growth.

The wound-healing experiments suggested that Myc might be required for hyperproliferative cycles. Epidermal hyperproliferation can also be found in the growing hair follicle. The proliferative marker keratin K5 was extensively expressed in follicular epidermis of control and KO mice of the same litter. However, there was loss of K5-positive cells in KO follicles (Fig. 6A). In addition, the hair follicle-hyperproliferative marker keratin K6 was significantly reduced in the Myc KO mice (Fig. 6A). The hyperproliferation defect might account for the hair alterations observed in KO mice. Sebaceous gland secretion, another skin function that is stimulated by Myc overactivation in the hair follicle (Waikel et al., 2001; Arnold and Watt, 2001), was also impaired in KO mice (Fig. 6B,C).

Myc has been proposed to have a role in keratinocyte stem cell differentiation, and this potential function may contribute to the phenotype observed in the KO mice. To investigate this issue we performed classical BrdU label-retaining experiments where the epidermis is extensively labelled in vivo in 10-day-
related transcription factor p63 (McKeon, 2004). Integrins (Jones and Watt, 1993) and more recently, the p53-pathway may have a role in ESC. These include the adhesion molecules β1 integrin and p63, considered a marker of human ESC but this is more controversial in mice. Deltap63 has been proposed to have a role in differentiation of ESC. Both β1 integrin and Deltap63 labelled the basal layer of young or adult control and KO epidermis (Fig. 7B). However, in KO epidermis the β1 integrin staining was generally weaker and more heterogeneous (Fig. 7B). Interestingly, Deltap63 expression was detected in groups of cells in the basal layer of control epidermis, but in frequent isolated cells in KO epidermis. The label retaining results and the expression of β1 integrin and p63 suggest that KO stem cells might divide more frequently and differentiate more rapidly (Fig. 9).

ESC give rise to transit amplifying cells (TAC) that undergo a rapid clonal expansion phase of active proliferation and subsequently enter terminal differentiation (Watt, 1989). Another keratinocyte hyperproliferative situation, where stem cells are overstimulated, is the primary stratified cultures in the presence of a fibroblast feeder layer, serum and growth factors (Barrandon and Green, 1987). To further study the effect of Myc inactivation on stem cell function, we performed clonogenicity assays in such conditions. In this assay, stem cells give rise to big, round, actively growing colonies, whereas cells in the clonal expansion phase (TAC) produce small, irregularly shaped and fully differentiated colonies (Jones and Watt, 1993). Control keratinocytes from adult normal littermates gave rise to both types of colonies, whereas these were absent in the KO cultures (Fig. 8A). When keratinocytes were isolated from newborns and a higher number of cells was plated, numerous tiny colonies were found in the KO cultures (red spots in Fig. 8B). These colonies consisted of two to four cells with a differentiated morphology that had smaller nuclei than controls (Fig. 8C). Thus, amplification of Myc KO stem cells did not occur in culture and instead, they seemed to undergo terminal differentiation after one or two cell divisions. To confirm this we followed the cell fate of freshly isolated keratinocytes plated at a higher density to favour proliferation, and in the presence of a feeder layer. KO keratinocytes never gave rise to colonies beyond four cells and all detected colonies were differentiated (Fig. 8D). However, some cell divisions and two-to-four cell colonies were still observed for 2 or 3 weeks, a period after which KO keratinocytes were no longer detected. BrDU incorporating KO keratinocytes were present after a week, but not after 3 weeks (Fig. 8D). These results were observed regardless of the age of the mouse, the level of expression of L-myc and whether or not it displayed a severe phenotype. Stem cell amplification was thus severely compromised in the KO cultures. Although we cannot rule out that the effect on the stem cells in the bulge of the hair follicle (Blanpain et al., 2004; Morris et al., 2004; Tumbar et al., 2004) might be different, as this issue was not within the scope of this study, altogether the results suggest that the stem cell compartment might be reduced in Myc KO epidermis.

**Discussion**

The results presented here show a central role for endogenous Myc in epidermal homeostasis. The reduction of the proliferative compartment led to premature differentiation both in vivo and in culture. However, keratinocytes were capable of cycling in vivo in the absence of Myc, and drastic cell-cycle

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**Fig. 6.** Proliferation in hair follicles and sebaceous function. (A) Hair follicle sections from control (Ct) or KO skin stained in red for keratin 5 (K5) or keratin 6 (K6) as indicated; nuclei were stained with DAPI (blue). Arrows indicate areas of loss of expression. (B) Control or KO skin stained in red for keratin 5 (K5) or keratin 6 (K6) as indicated; nuclei were stained with DAPI (blue). Arrows indicate areas of loss of expression. (C) Oil red O staining on sections from back skin to detect sebaceous secretion. Arrowheads point at sebaceous glands. Every study was carried out with mice of the same litter. Bar, 20 µm.
phasing defects were not observed in proliferative cells, in agreement with observations in Drosophila mutants (Johnston et al., 1999). It is worth noting, though, that an upregulation of L-myc was detected in younger mice before the more severe phenotype arose. A possible compensatory effect by L-myc or other pathways supporting proliferation in KO epidermis, cannot be ruled out. However, keratinocytes continued to cycle in adult mice even when L-myc and B-myc were downregulated and N-myc was undetectable. In addition, neither a compensatory effect nor the presence of cells carrying undeleted Myc was detected in culture, as no growing clones were obtained from any KO mouse. Nevertheless, the hyperproliferation involved in wound healing, hair follicles and stratified cultures was consistently and severely compromised, indicating that Myc is required for rapid epidermal proliferative cycles.

Myc, cell size and endoreplication
KO cells were significantly smaller, suggesting that cellular growth might be the primary defect in keratinocytes without Myc, causing the observed effects on the proliferation/differentiation balance. Suppression of Myc activity in epidermis also impaired keratinocyte endoreplication. Endoreplication has been often disregarded or poorly studied in mammalian organisms, in spite of the fact that it is found in various human tissues including epidermis (Gandarillas et al., 2000) (J.Z. et al., unpublished), and that it might have an important role in cellular growth (for example, see Edgar and Orr-Weaver, 2001). A correlation between DNA ploidy, cell-size and differentiation has been observed in plants, Drosophila and mammals, but their interdependence is not clear. However, it has been shown that endoreplication allows plant cells to increase in size, also during epidermal differentiation (Traas et al., 1998; Kondorosi et al., 2000). Our present data suggest that endoreplication is not required for terminal differentiation to take place, but that it is necessary for the cell enlargement that occurs during normal post-mitotic differentiation (Banks-Schlegel and Green, 1981; Gandarillas et al., 2000).

Myc and stem cell function
A possible role of Myc in regulating stem cell biology has been recently proposed (Gandarillas and Watt, 1997; Waikel et al., 2001; Arnold and Watt, 2001). Our data show that Myc KO stem cells are unable to amplify and have a limited life span in culture, and give rise to a limited proliferative progeny in vivo. Reconciling the data reported for the exogenous and the endogenous gene on keratinocytes reveals that Myc pushes stem cells into clonal expansion along the differentiation programme when overactivated (Gandarillas and Watt, 1997; Waikel et al., 2001; Arnold and Watt, 2001), but causes stem cell daughters to enter premature differentiation when absent. Altogether our results suggest that the stem cell turnover is higher and that their compartment might be reduced, in adult Myc KO epidermis. A logical rationale to explain this seems that incapable to undergo rapid amplification and in order to sustain epidermis, stem cells are forced to divide and undergo differentiation more frequently and therefore, their compartment might be progressively reduced. This would explain why the difference observed in the replication index between KO and control epidermis at a given time was not drastic and yet the proliferative compartment (TAC) was reduced. According to this, Myc would not be required for stem cell divisions, nor ‘a switch’ of the stem cell decision to enter the differentiation programme (Gandarillas et Watt, 1997), but it would be ‘an amplifier’ of such a decision (Fig. 9). If this model is correct, the skin should have more difficulties to grow with time and indeed, a more severe phenotype appeared after 2 or 3 months of age. Another expectable consequence of forcing stem cells to divide more frequently is that the epidermis would age prematurely, and this seems to be the case of adult KO skin, since it appeared atrophic and slow regenerating. Altogether, a correct Myc activity appears essential for normal stem cell function and renewal.

Myc and cell growth
The tight and shiny skin and the reduced body size of epidermal Myc KO mice resemble the phenotype of epidermal-specific transgenics for the keratinocyte growth-inhibitory factor TGF-β (Sellheyer et al., 1993), which is known to
downregulate Myc. These features are also reminiscent of the ‘tight skin contracture’ human inherited lethal syndrome affecting newborns (Lowry et al., 1985; Lenz and Meschede, 1993; Abrahamson and Stone, 2002). Interestingly, this condition has been described by some authors as ‘generalised skin atrophy’ or hypoplasia (Lenz and Meschede, 1993), and this is consistent with our observations.

A key remaining question is whether mammalian Myc function and cell growth are coupled to cell division. Johnston et al. (Johnston et al., 1999) concluded that Drosophila dmyc stimulates the G1/S transition independently of mitosis control, thus resulting in increased cell size. We made similar observations after constitutive activation of Myc in human keratinocytes, which resulted in cell size increase upon a cell division block (Gandarillas et al., 2000). This coincidence is consistent with the fact that dmyc and Myc share biological activities (Gallant et al., 1996; Schreiber-Agus et al., 1997). However, by reducing Myc expression in the whole mouse body, Trumpp et al. (Trumpp et al., 2001) found fewer cells of normal size in some organs. They suggested that Myc is not essential for cellular growth and instead controls the decision of cells to divide or not to divide, due to a tighter coupling of cell growth and cell division in mammals. Our results show that endogenous Myc controls mammalian epidermal cell size both in proliferative and especially, in postmitotic cells. Two possible reconciling explanations for such discrepancy are that the mice analysed by Trumpp et al. had some remaining Myc expression, and/or that the consequences of Myc function are tissue dependent and cell context dependent rather than species specific. Interestingly, Myc produced larger cells when overexpressed (Kim et al., 2000) and smaller cells when inactivated (Baena et al., 2005), in mouse liver, another endoreplicative tissue. Interestingly, two different groups have reported a requirement of dmyc in Drosophila endoreplication (Maines et al., 2004; Pierce et al., 2004). A selective advantage of cells overexpressing dmyc in Drosophila has also been recently reported by inducing apoptosis of cells with lower levels of dmyc (de la Cova et al., 2004; Moreno and Basler, 2004). Whether this effect is related or not to cell growth
remains to be elucidated. A role for Myc in stem cell renewal has also been very recently reported by Wilson et al. (Wilson et al., 2004) in bone marrow. In agreement with our observations, the authors conclude that Myc is not required for stem cell proliferation, although in this case they find an accumulation of stem cells. Different tissue-regulatory mechanisms and cell contexts might account for the different consequences of Myc function (a similar conclusion has been drawn from overexpression of Myc in mouse liver by Beer et al.) (Beer et al., 2004).

The study presented here shows that at least in some tissues and as for dmyc in flies, mammalian Myc function and cell growth are not tightly coupled to cell division. This implies that in addition to cell growth control deregulation, oncogenic alterations may have to hit the mitosis checkpoint to trigger tumorigenesis. In all cases reported, nevertheless, endogenous or exogenous Myc function resulted in increased biomass production, an advantage that tumours would clearly not hesitate to select. Unifying Myc functions in the mammalian body and carcinogenesis should now be the challenge.

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