Something to SNF about

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Factors that modify chromatin are crucial for regulating gene expression, but what, in turn, regulates these factors? A new study highlights the importance of signaling cascades in recruiting chromatin-remodeling enzymes to specific promoters during muscle differentiation.

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Skeletal muscle differentiation relies on signals that induce the transcriptional activity of MyoD, a basic helix-loop-helix transcription factor that acts as a key regulator of the muscle determination program. Muscle differentiation also depends on chromatin-modifying factors that acetylate and remodel histones to unravel chromatin and allow further access of transcriptional machinery to promoters[1]. The differentiation-activated p38α/β signaling pathway also stimulates the transcriptional activity of MyoD[2], but it was not clear whether this occurred directly, through MyoD phosphorylation, or indirectly, perhaps through chromatin modifiers. On page 738, Simone and colleagues[3] show that p38α/β signals directly to the SWI-SNF chromatin remodeling complex, thereby recruiting SWI-SNF to specific target promoters, where it cooperates with MyoD to activate transcription of muscle-specific genes.

Modifying chromatin

Previous work showed that inhibiting the p38α/β pathway with the drug SB203580 prevents myoblast differentiation due to the inability of MyoD to activate transcription[2,4,5]. To further clarify the mechanism and test the hypothesis that p38α/β indirectly regulates MyoD activity, Simone *et al.* examined the effects of SB203580 on chromatin modifications at the promoters of the differentiation genes myogenin (*Myog*) and muscle creatine kinase (*Ckm*). They found that the acetylation status of histones H3 and H4 was unaltered by inhibition of p38α/β signaling. Consistent with this, the interaction between MyoD and the histone acetyltransferases p300 and PCAF[6] still occurred at these promoters in the absence of p38α/β signaling. These results suggest that, even when functional MyoD-acetyltransferase complexes are present on these promoters, activation of gene expression requires a further signal from p38α/β.

MyoD contains two domains that mediate the remodeling of chromatin[7], and SWI-SNF chromatin remodeling activity is also necessary for MyoD-mediated differentiation[8]. Simone *et al.* therefore tested whether p38α/β signaling affects chromatin remodeling at the *Myog* and *Ckm* promoters. Indeed, they found that chromatin was not remodeled in the absence of p38α/β signaling, suggesting several possibilities: (i) p38α/β signals directly to MyoD to activate its intrinsic chromatin remodeling activity; (ii) p38α/β signaling recruits the SWI-SNF complex to promoters; (iii) p38α/β activates the SWI-SNF complex already present on promoters; or (iv) some combination of the above.

Targeting SWI-SNF

To narrow down the possibilities, Simone *et al.* examined the association of BRG1 and BRM, the ATPase subunits of the SWI-SNF complex, with the *Myog* and *Ckm* promoters. They found that inhibition of p38α/β prevented the association of BRG1 and BRM with MyoD, implying that p38α/β signaling is involved in recruiting the SWI-SNF complex to specific myogenic promoters. They also showed that p38α/β phosphorylates the BAF60 subunit of the SWI-SNF complex *in vitro*, suggesting a direct link between p38α/β and SWI-SNF activity.

These studies show that blocking p38α/β activity results in a failure to recruit the SWI-SNF complex to myogenic promoters. What happens when myoblasts are forced to differentiate by the overexpression of the upstream p38α/β activator MKK6? Simone *et al.* found that forced activation of the p38α/β pathway in myoblasts resulted in premature association of MyoD with BRG1, p300 and PCAF on the *Myog* promoter, with only the MyoD-BRG1 association dependent on p38α/β signaling.

To determine whether p38α/β signals directly to MyoD in addition to the SWI-SNF complex, Simone *et al.* transfected SW13 cells deficient in BRG1 and BRM with constructs expressing Gal4 fused to full-length MyoD or to a truncated form of MyoD lacking the chromatin-remodeling domains. They found that neither Gal4 fusion protein

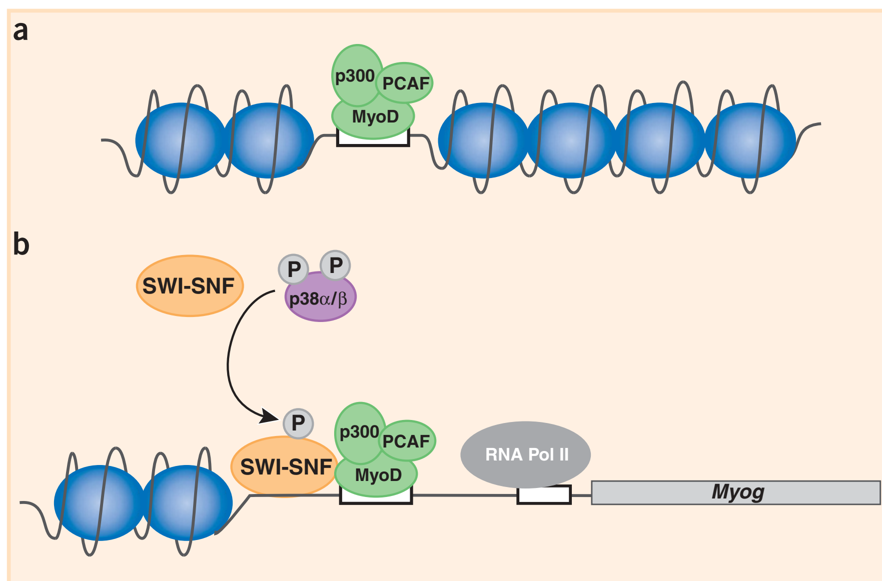

Figure 1 Regulation of MyoD-dependent gene expression by p38α/β. (**a**) The MyoD-p300-PCAF complex is bound to DNA but unable to activate transcription in the absence of further signals. (**b**) p38α/β targets SWI-SNF to the MyoD-p300-PCAF complex, resulting in remodeling of chromatin at the *Myog* and *Ckm* promoters and activation of gene expression.

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could activate a Gal4-dependent reporter further in response to MKK6-induced p38α/β signaling. In fact, further activation of the reporter was not observed until either BRG1 or BRM was added back to these cells. These results provide compelling evidence that p38α/β does not signal directly to MyoD or affect its intrinsic remodeling activity.

Mef2 proteins cooperate with MyoD to induce gene expression[9] and are also targets of p38α/β signaling[2,5,10]. Could Mef2 proteins be mediating some of the observed effects? Notably, the truncated version of MyoD, which lacks the ability to associate with Mef2 (ref. 11), retains the ability to activate the reporter in the presence of BRG1 or BRM and p38α/β signaling. These findings argue against an essential role for Mef2 under the assay conditions used, but further studies are required to examine this question in detail.

Pathways to activation

This study by Simone *et al.* provides evidence that the p38α/β pathway promotes muscle differentiation by indirectly signaling to MyoD through the SWI-SNF chromatin-remodeling complex, resulting in expression of *Myog* and *Ckm* (**Fig. 1**). Increasing evidence over the past few years suggests that, in addition to targeting transcription factors, kinases can alter gene expression by targeting chromatin-modification factors. Simone *et al.* have demonstrated this for the first time with the p38α/β pathway in muscle.

As p38α/β regulates only a small proportion of MyoD target genes[12], it will be interesting to determine if other promyogenic signaling molecules are responsible for recruiting chromatin modifiers to different loci. It will also be important to determine exactly how BAF60 phosphorylation influences the selective recruitment of SWI-SNF to specific target loci. Are all p38α/β regulated targets in muscle activated by this mechanism, or do Mef2 proteins mediate some of the observed effects of p38α/β signaling on muscle-specific transcription? Finally, it will be important to determine how this signaling mechanism influences muscle formation *in vivo*. Mutating the phosphorylated residue(s) of BAF60 or the residue(s) important for the interaction between BRG1 and MyoD, and targeting these mutants to endogenous loci in mice, could be used to determine the importance of this signaling mechanism in regulating the muscle differentiation program.

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From proteomics to disease

Kenneth H Kraemer

The nucleotide excision repair system is essential for repairing DNA damage caused by exposure to sunlight. Now, parallel studies in yeast and individuals with a rare disease called trichothiodystrophy have identified a new component of the DNA repair and basal transcription factor TFIIH.

In 1968, James Cleaver described a defect in DNA repair in cells from three individuals with a rare disease called xeroderma pigmentosum[1] (**Fig. 1**). The DNA repair defect in these individuals resulted from a failure of the nucleotide excision repair (NER) system to remove photoproducts from DNA that is damaged by ultraviolet (UV) radiation. Individuals with xeroderma pigmentosum develop pigmentary abnormalities on their skin at an early age and have 1,000 times more sunlight-induced cancers of the skin and eyes[2]. Organisms as diverse as yeast and humans have NER systems composed of homologous proteins that function together to recognize DNA damage, unwind DNA in the damaged region, excise the damage to create a gap and fill in the gap using the undamaged strand as a template (**Fig. 1**)[2,3]. Defects in seven of these proteins (XPA–XPG) are associated with xeroderma pigmentosum in humans.

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In 1993, a group led by Jean-Marc Egly, working to define the basal transcription factor TFIIH, reported that two TFIIH components were also DNA repair proteins: XPB and XPD[4,5]. In this issue, Jeffrey Ranish and colleagues[6] and Giuseppina Giglia-Mari and colleagues[7] report a new shared component of the DNA repair and basal transcription machinery, called TFB5, which is linked to a rare human disease called trichothiodystrophy (TTD).

A crucial link

The observation of Egly and colleagues established a crucial link between transcription and DNA repair, which explained why mice completely lacking XPB or XPD showed embryonic lethality, as TFIIH is essential for survival. Nevertheless, certain mutations in *ERCC3* (encoding XPB) and *ERCC2* (encoding XPD) are present in individuals with xeroderma pigmentosum and are compatible with survival[8]. There are varied clinical phenotypes associated with different defects in XPD and XPB in humans (**Fig. 1**). Some involve progressive neurologic degeneration, and others involve short stature, developmental delay and severe wasting (the xeroderma pigmentosum and Cockayne syndrome complex). These distinct manifestations seem to reflect defects in different functions of the same protein.

Several years ago, Miria Stefanini and colleagues observed that some individuals with defects in XPD or XPB have a phenotype known as TTD[9–11]. Cells from these individuals behave in culture like those of individuals with xeroderma pigmentosum, but the affected individuals themselves present a very different phenotype characterized by sulfur-deficient brittle hair, skin photosensitivity without increased pigmentation and