Thomas Braun - The function of transcriptional co-regulators for MCAT-dependent muscle-specific gene activity during development and disease

Background
Transcriptional control of skeletal muscle-specific gene expression is achieved by combined action of various transcription factors that are either specifically expressed in the skeletal muscle lineage or show a more widespread expression pattern. Several groups of transcription factors such as the MyoD (Neuhaus and Braun, 2002), MEF-2 (Black and Olson, 1998) and TEF-families of DNA-binding proteins (Larkin and Ordahl, 1999), which bind E-boxes and muscle-specific CAT (=MCAT) elements, have been identified by different means to play important roles for the development of muscle tissues and for the regulated expression of muscle specific genes. It seems rather easy to anticipate how tissue-specific regulation of muscle cell-specific genes might be achieved by transcription factors that are themselves tissue-specific such as the MyoD family of transcription factors (Braun et al., 1992; Braun et al., 1990; Weintraub et al., 1991). A variation of this theme is a combination of basal factors with tissue-specific transcription factors or an exclusive blend of transcription factors, which are not unique as individual molecules in a given tissue but exclusive in respect to their joint expression profiles. A seeming paradox to this rule is the finding that individual DNA binding sites, which bind widely expressed transcription factors such as serum response factors (SRF) (Carson et al., 1996) and transcriptional enhancer factors (TEF), are able to confer muscle specific expression of individual genes. Examples comprise the α-skeletal actin and cardiac TroponinT genes, which depend on SRF and TEF transcription factors, respectively. In such cases muscle specific transcription might be achieved by the assembly of larger transcriptional complexes, which utilize DNA-binding proteins as docking stations for tissue-specific bridging or co-activating factors that might confer additional activities to DNA-bound proteins (McKenna and O'Malley, 2002). Recently, we have identified a new family of transcriptional co-regulators (VITO gene family) (Mielcarek et al., 2002), which bind to different TEF transcription factors (Gunther et al., 2004). At least two members of this family, which is characterized by the presence of a SID (scalloped interaction domain) are specifically expressed in the skeletal muscle lineage and are up-regulated in differentiating muscle cells (VITO-1 and VITO-2, unpublished). VITO-1 and VITO-2 are part of a larger protein complex that comprises of TEFs, YAP65, and other yet unknown proteins ((Vassilev et al., 2001) and unpublished results) and confer a strong transactivation capacity to TEFs.

Aims
We have inactivated the VITO-1 gene in mouse ES-cells and are currently breeding homozygous mutants to analyze its role for muscle cell differentiation and regeneration. Since VITO-1 and VITO-2 are expressed in an overlapping pattern during myogenic development (Mielcarek et al., 2002) we will generate VITO-2 mutants and compound mutant animals. In addition, we will analyze the biochemical functions of VITO family members and characterize additional binding partners of VITO and TEF proteins by mass spectrometry, a TAP tag strategy, and genetic complementation techniques. Similar techniques will be used to study the composition of the TEF/VITO/YAP65 complex. We will also investigate the role of posttranslational modifications (i.e. acetylation) for the activity of TEF/VITO/YAP65 complex using Sirt7/sirtuin (a protein deacetylase) mutant mice, which we have generated recently.
Preceding studies have shown that a constitutive TEF-1 knockout is lethal (Chen et al., 1994). Therefore, we will establish a conditional TEF-1 knockout strain and, in a later stage of the project, mutant mouse strains for TEF-3 and TEF-5, which are also expressed in striated muscle albeit at different developmental stages. To achieve muscle specific gene ablation we will use an array of transgenic and knockin mouse strains established in our lab (MyHC-Cre, Myf5-Cre, Desmin-Cre, MCK-Cre, MyLC-Cre) (Kaul et al., 2000). These approaches will allow us to answer the following specific questions:

1. Does the transcriptional activity of TEF-1 in muscle cells depend on VITO-1 or VITO-2?
2. What is the specific role of individual components of the TEF/VITO/YAP65 complex for muscle specific gene activation? Does the TEF/VITO/YAP65 complex contain additional proteins and do they affect DNA binding specificity and/or transactivation?
3. Does the up-regulation of VITO-1 in certain disease models of heart muscle failure (MyHC-MCP1, SOD conditional knockouts, isoproterenol induced cardiac hypertrophy) reflect a protective reiteration of developmental mechanisms to achieve adaptive remodelling or does an aberrant expression of these molecules contribute to disease processes?
4. Are TEF-transcription factors and/or their co-activators indispensable for myogenic differentiation or do they merely control expression of a selected set of cell-type specific genes?
5. How does the composition of the TEF/VITO/YAP65 complex differ between cardiac and skeletal muscle cells?

Cited literature illustrating background


Own publications (last five years)


Adamska, M., Herbrand, H., Adamski, M., Krüger, M., Braun, T., and Bober, E. (2001). FGFs control the patterning of the inner ear but are not able to induce the full ear program. Mech. Dev. 109, 303-313.


