



Lincoln's Inn Fields

## Signalling and Transcription

[www.london-research-institute.org.uk/richardtreisman](http://www.london-research-institute.org.uk/richardtreisman)

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Changes in cell identity, proliferation and behaviours such as motility and adhesion are induced by extracellular signals which in many cases act by changing patterns of gene transcription. We study a network of transcription factors involved in all these processes, which controls the activity of the MADS-box transcription factor SRF (Serum Response Factor). Work this year has given new insights into molecular mechanism of actin-MAL interaction and functional redundancy amongst TCF family members, and has demonstrated that MRTF activity is required for numerous cellular behaviours that depend on actin cytoskeleton remodelling.

SRF interacts with members of two families of regulatory cofactors, which contact both its DNA-binding domain and the neighbouring DNA. The TCFs (Ternary Complex Factors: Elk-1, SAP-1, Net) are Ets domain proteins that link SRF activity to MAPK signalling pathways, while the MRTF (Myocardin-related Transcription Factors: MAL and Mkl2) link its activity to the dynamics of the actin cytoskeleton.

#### Progress in understanding regulation of MAL by actin

Rho GTPase signalling controls the activity of the SRF

network indirectly, by altering G-actin levels, which in turn appears to control the formation of actin-MRTF complexes. Central to this regulation is the highly conserved RPEL domain at the MRTF N-termini which contains three so-called RPEL motifs. With Stephane Mouilleron and Neil McDonald (LRI Structural Biology Group), Sebastian Guettler previously solved the structures of cocrystals between actin and either RPEL peptides or RPEL domain subfragments. The RPEL motif-actin interaction shares features with other actin-protein complexes and exhibits a striking similarity to the interactions with actin made by two discrete regions of vitamin D binding protein. Carola Langer used site-directed mutagenesis to verify that the interactions seen in the structure are functionally relevant. With Maria Wieszlak, Carola is working to examine the relevance to MAL regulation of larger actin-RPEL complexes, whose structures are currently under investigation.

Tamara Gruener has developed high throughput RNAi screens for the genetic identification of MRTF regulatory factors in drosophila tissue culture cells, in which the MRTF-SRF pathway is conserved. Working with Mike Howell in the LRI high-throughput screening facility, Tamara has identified a number of candidate regulatory factors in this screen, many of which are conserved through vertebrate evolution. The majority of these proteins appear to affect reporter activity but not MRTF localisation, but several appear to act at the level of MAL nucleocytoplasmic shuttling. Tamara is currently investigating the mechanism by which these proteins affect MRTF activity. Rafal Pawlowski has also continued studies on the role of phosphorylation in MAL regulation using phospho-specific antibodies and mass spectrometry. His data suggest that phosphorylation plays both positive and negative roles in transcriptional activation by MAL but does not affect its nucleocytoplasmic shuttling.

### SRF-cofactor interactions

Classical studies in tissue culture cells suggested that immediate-early gene activation plays an essential role in cell cycle re-entry function, and immune cells in our TCF knockout animals provide an interesting system in which to study this. Patrick Costello and Diane Maurice find that while IE gene expression appears to sensitise some cell types to cell cycle re-entry in response to TCR ligation, in others it appears to inhibit it. Diane is currently investigating the molecular basis of these phenomena.

Cyril Esnault has worked with Rob Nicolas to develop chromatin immunoprecipitation approaches for the study of SRF-cofactor interactions in fibroblasts and immune cells, aiming to investigate the factors which determine specificity of cofactor recruitment for different SRF target genes. These methods will be used on a genome-wide scale to investigate cofactor activity during growth factor stimulation. Rob Nicolas' array analysis of gene transcription following TCR activation in DP thymocytes demonstrated that the classical SRF-controlled IE genes are differentially sensitive to TCF deletion; with Cyril Esnault he has shown that this apparently reflects differential TCF recruitment to specific SRF target genes. Differential TCF recruitment may underlie Patrick and Rob's finding that ectopic expression of Elk-1, but not Net, can rescue the thymocyte selection defect in animals lacking SAP-1. Anastasia Mylona has used SRF mutants to demonstrate that cofactor interactions are essential for T cell development, and is continuing this work by examining cofactor interactions using biochemical approaches.

### The SRF network in cytoskeletal dynamics and metastasis

Rho GTPases control the dynamics of the cytoskeleton through multiple effector proteins that regulate the assembly, activity and turnover of actin-based structures, thereby controlling cell adhesion, morphology and motility. Numerous studies of both human cancers and mouse cancer models have implicated Rho signalling, and RhoC in particular, in cancer cell invasion and metastatic tumour spread, and this has been generally thought to reflect direct effects on the cytoskeleton. Although the control of MRTF activity by Rho potentially provides a mechanism by which cytoskeletal gene expression can be coordinated with cytoskeletal regulation, the notion that transcriptional regulation via the Rho-actin-MRTF-SRF nuclear signalling pathway is required in addition to Rho-controlled cytoplasmic events for effective execution of cytoskeletal remodelling has remained untested.

To address these issues directly, and to elucidate its significance for tumour metastasis, Souhila Medjkane and Cristina Perez studied the role of MRTF-SRF signalling in two highly metastatic tumour cell lines, human MDA-MB-231 breast carcinoma and mouse B16F2 melanoma. Depletion of MRTFs or SRF using shRNA-mediated knockdown reduces cell adhesion, spreading, invasion and motility in culture,

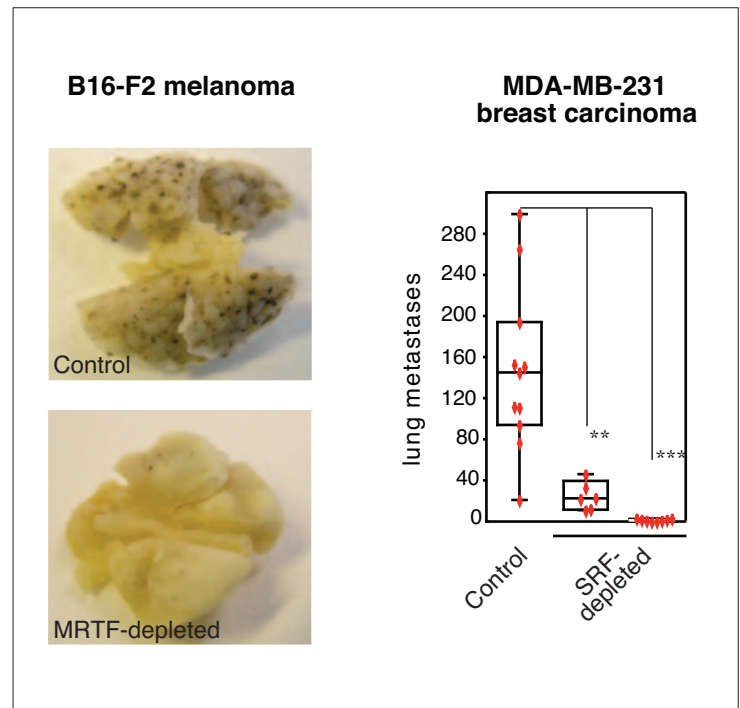


Figure 1. MRTF-SRF signalling is required for experimental metastasis. Left, B16F2 mouse melanoma colonies established in the mouse lung by control and MRTF-depleted cells are visible as dark spots. Right, human MDA-MB-231 breast carcinoma cells depleted of SRF are greatly impaired in lung colonisation compared to control cells, as assessed following H+E staining of lung sections.

without affecting proliferation or inducing apoptosis. In studies carried out with Cedric Gaggioli and Erik Sahai, Souhila found that MRTF-depleted tumour cells were strikingly impaired in organotypic invasion models, and exhibited reduced cell motility in tumour xenografts, suggesting a defect in metastatic potential. Consistent with this, MRTF- and SRF-depleted tumour cells are unable to colonise the lung from the bloodstream in an experimental metastasis model, even though they initially arrive at the organ in similar numbers to non-depleted cells (Figure 1). Actin-based cell behaviour and experimental metastasis thus requires Rho-dependent nuclear signalling through the MRTF-SRF network. Microarray analysis identified *Myh9* and *Myl9* as among a small number of shared MRTF-SRF targets required for these processes. Jonathan Tobin and Victoria Lawson will take this work forward to investigate at which steps MRTF-SRF signalling is involved in the metastatic process *in vivo*.

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