



Lincoln's Inn Fields

Mammalian Genetics

www.london-research-institute.org.uk/axelbehrens

Group Leader **Axel Behrens**

Associate Scientist

Nnennaya Kanu

Postdoctoral Scientists

Cristina Aguilera

Atanu Chakraborty

Janet Cronshaw

Clare Davies

Xavier Fontana

Joanna Loizou

Rocio Sancho

Javier Villadiego

Graduate Students

Joerg Hoeck

Kay Penicud

Scientific Officer

Clive Da Costa

Cellular responses to extracellular signals are mediated by changes in transcription factor activity and subsequent modulation of target gene expression. Mitogen activated protein (MAP) kinases and the DNA damage kinase ATM (**A**taxia **T**elangiectasia **M**utated) are essential mediators of signal transduction and transcriptional control in eukaryotic cells. Transcriptional regulation by MAP kinases and ATM is causally involved in a number a clinically relevant human diseases, including neurodegeneration and cancer. Our major focus is the elucidation of the molecular mechanism of MAP and ATM kinase signalling and to understand their function in disease.

Regulation of intestinal homeostasis and tumourigenesis by JNK signalling

Wnt signalling is a key signalling pathway controlling intestinal homeostasis and cancer. We have recently found that the Jun N-terminal kinase (JNK) MAP kinase pathway and one of its most important substrates, the AP-1 transcription factor c-Jun, modulates Wnt signalling strength in the intestine.

Transgenic gut-specific augmentation of JNK signalling stimulated progenitor cell proliferation and migration, resulting in increased villus length. In the crypt, c-Jun protein was highly expressed in intestinal stem cells (ISCs) (Figure 1a) and absence of c-Jun resulted in decreased proliferation and villus length. In addition to several known c-Jun/AP-1 target genes, expression of Wnt targets genes Axin2 and Lgr5 were stimulated by JNK activation, suggesting a crosstalk of JNK to Wnt signalling. Expression of the Wnt pathway component TCF4 was controlled by JNK activity and chromatin immuno-precipitation and reporter assays identified *tcf4* as a direct c-Jun target gene. Consequently, increased JNK activity accelerated tumourigenesis in a model of colorectal carcinogenesis. Since *c-jun* is a direct target of TCF4/ β -catenin, the control of *tcf4* expression by JNK/c-Jun leads to a positive feedback loop that connects JNK and Wnt signaling (Figure 1b). This mechanism regulates the physiological function of progenitor cells and oncogenic transformation.

Molecular mechanism of phosphorylation-dependent c-Jun-mediated neurodegeneration and cancer

AP-1 activity is strongly induced in response to numerous signals including growth factors, cytokines and extracellular stresses. AP-1 stimulation is mediated, in part, by the phosphorylation of c-Jun by the JNKs. c-Jun N-terminal phosphorylation (JNP) at the serine residues 63 and 73 and threonine residues 91 and 93 within its transactivation domain is thought to increase transcription of target genes, one of which is the *c-jun* gene itself. Genetic inactivation of c-Jun N-terminal phosphorylation by a knock-in approach demonstrated an essential role for JNP in stress-induced neuronal apoptosis and tumorigenesis. As the JNK/c-Jun pathway is required for clinically important diseases including neurodegenerative insults and cancer, mediators of JNP may be promising candidates for therapeutic intervention.

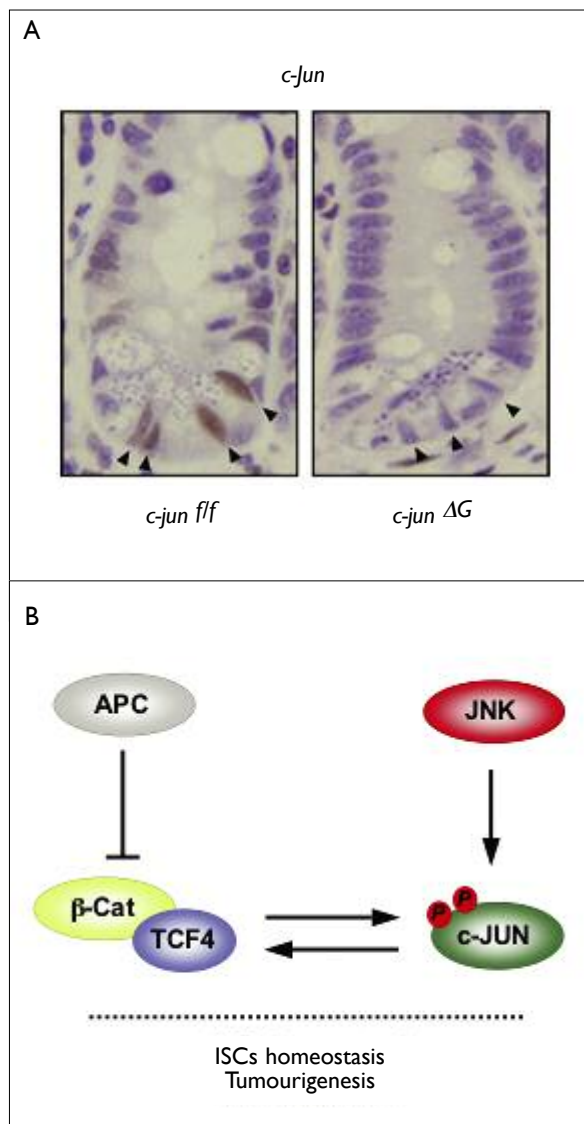


Figure 1. Function of JNK/c-Jun in intestinal stem cells and cancer. a) c-Jun protein is expressed in ISCs (indicated by arrowheads) and staining is absent in gut-specific c-jun knock-out mice (*c-jun^{ΔG}*). b) Model illustrating a positive feedback loop that connects JNK and Wnt signaling.

To investigate the mechanism of JNK-mediated transcriptional regulation, we have identified proteins that interact with c-Jun in a phosphorylation-dependent manner. Several candidate proteins that preferentially interact with N-terminally phosphorylated c-Jun (Phosphorylation-dependent c-Jun interactor (PDJ) 1, 3, 4 and 5) or that bind preferentially to the unphosphorylated form of c-Jun have been identified (PDJ2).

We have recently shown that PDJs are novel disease-relevant regulators of the JNK pathway. For example, PDJ3 encoded the tumor suppressor Fbw7, and Fbw7 was found to be an E3 ubiquitin ligase specific for phosphorylated c-Jun. Another PDJ encoded the transcription factor TCF4 and our studies revealed that the interaction of phosphorylated c-Jun with TCF4 is essential for intestinal cancer development.

Preliminary characterisation revealed that other PDJs have several different biological activities, ranging from transcriptional cofactors to chromatin regulators. Currently we are using biochemical methods and gene targeting in mice to understand the functions of PDJs in neurodegeneration and cancer. The combination and integration of the biological activities of phosphorylation-dependent interactors of c-Jun have the potential to elicit basically any physiological response depending on the cellular context and could be an important mechanism underlying cell-type specificity of MAP kinase signalling.

ATMIN defines a novel pathway of ATM signalling

ATM is a member of the phosphatidylinositol kinase-related protein family that includes ATR and DNAPKcs. These kinases respond to the presence of DNA damage or replication blocks by activating cell cycle checkpoints and promotion of DNA repair. ATM is mutated in the genomic instability syndrome ataxia telangiectasia (A-T), which is characterised by problems in motor coordination, immunodeficiency and increased tumour incidence. We have recently identified a novel essential cofactor for ATM, which we named ATMIN (for *ATM interacting protein*). ATMIN interacts with ATM through a carboxy-terminal motif, which is also present in another ATM cofactor called Nijmegen breakage syndrome 1 (NBS1). ATMIN and ATM colocalised in response to ATM activation by chloroquine and hypotonic stress, but not after induction of double-strand breaks by ionizing radiation (IR). ATM/ATMIN complex disruption by IR was attenuated in cells with impaired NBS1 function, suggesting competition of NBS1 and ATMIN for ATM binding. ATMIN protein levels were reduced in A-T cells and ATM protein levels were low in primary murine fibroblasts lacking ATMIN, indicating reciprocal stabilisation. While phosphorylation of Smc1, Chk2 and p53 was normal after IR in ATMIN-deficient cells, basal ATM activity and ATM activation by hypotonic stress and inhibition of DNA replication was impaired. Thus ATMIN defines a novel NBS1-independent pathway of ATM signalling.

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