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NHS Cambridge University Hospitals NHS Foundation Trust

CRUK Cambridge Centre MRes rotation project

Rotation Project Title	Do nucleolar functions underpin the oncogenic potential of the tyrosine kinase, ACK?
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Programme	Cellular and Molecular Biology
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Laboratory Location	Department of Biochemistry (Sanger Building)

Project Outline	<u>**Aims and objectives**</u> The tyrosine kinase ACK is a downstream effector protein for the small G -protein Cdc42. ACK is a driver of oncogenic transformation, however relatively little is known about its cellular targets and/or partners. Using a yeast-2-hybrid screen, we identified 14 novel ACK partners, many of which are implicated in cancer-associated pathways. Two proteins we identified were the dead box protein DDX49 and a transcription regulator MCRS1. DDX49 was discovered in a survey of human nucleolar proteins and has no assigned function. However DEAD box proteins are often active in metabolic processes involving RNA and in fact many are RNA helicases. We have now shown that DDX49 binds ATP and both poly G and poly C beads, indicating that it is likely to bind RNA.The yeast homologue of DDX49 is a protein called Dbp8 which is involved in the very early stages of processing of precursor rRNA. We have observed ACK in the nucleus of cells and are investigating important nuclear roles for ACK in the cell. We have also shown that ACK phosphorylates DDX49. Our aim is to confirm the role of DDX49 in human cells and uncover how ACK phosphorylation impinges on DDX49 function. MCRS1 has many and varied functions but one, interestingly, is in the regulation of rRNA transcription. In the nucleolus MCRS1 associates with Mi2β and RFP and activates rRNA production via RNA polymerase1. It therefore appears that these two novel interacting partners for ACK, potentially control different aspects of rRNA levels: MCRS1 in increasing rRNA transcription levels and DDX49 likely in the early stages of rRNA processing, indicating that there are two nodes for ACK to regulate ribosome production. It has been known for decades that cancer cells, and indeed just actively proliferating cells, possess more and larger nucleoli and it is intuitive that they would need more ribosomes. We predict that ACK is acting via DDX49 and MCRS1 to increase ribosome biogenesis in cancer cells. This project will increase our understanding of the oncogenic signaling network that is regulated by ACK.
Experimental plan	We will start by identifying the phosphorylation site(s) for ACK on DDX49. We will mutate the 7 tyrosines in DDX49 to phenylalanine and monitor the ability of ACK to phosphorylate DDX49 variants. Identification of the target site on DDX49 for ACK will indicate the likely function/outcome of the interaction. Currently we suspect ACK does not phosphorylate MCRS1. We have however observed that DDX49 and MCRS1 interact with each other suggesting that they may form part of a larger complex which is capable of integrating the regulation of ribosomal rRNA biogenesis by co-ordinating rRNA transcription and processing of precursor rRNA. We will investigate whether ACK is regulating the DDX49/MCSR1

	interaction by co-expression and co-immunoprecipitation. MCRS1, in complex with Mi-2β/RFP/UBF, up-regulates ribosomal gene transcription. We have reporter plasmids that allow us observe MCRS1 transactivation of the rDNA promoter. We will monitor ACK effects in this system. We will also pursue the possibility that the ACK/DDX49, ACK/MCRS1 and DDX49/MCRS1 interactions function to localize the proteins correctly. We have observed that ACK affects the cellular localization of other interacting partners. We will determine the cellular localization (using sub-cellular fractionation) of the proteins alone, in complex and with different upstream stimuli. We have also observed that ACK plays a role in regulating the protein levels of some of its interacting partners. We will determine the stability of DDX49 and MCRS1 using cycloheximide and the see if ACK regulates degradation of either. We hypothesize that DDX49/MCRS1 function may underpin the tumourigenic potential of ACK. We have shown ACK enhance cell proliferation and so will use siRNA to investigate the role DDX49/MCRS1 plays downstream of ACK, in cell growth. Experiments carried out in the initial 15-week rotation would naturally progress into a full PhD project directed by the preliminary results generated.
Main Techniques	• Molecular Biology • Cell culture • Western blotting • Co-immunoprecipitation • Cell fractionation
Key References	Mahajan, K. and Mahajan, N.P. Oncogene (2015) 34: 4162 Grimwood, J. et al. (2004) Nature 428:529 Daugeron, M. L. and Linder, P. (2001) Nucl. Acids Res. 29:1144 Hirohashi, Y. et al. (2006) Oncogene 25:4937