Investigating the role of Histone Deacetylase HDAC4 in long-term memory formation

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ABSTRACT

Epigenetic mechanisms are emerging as master regulators of cognitive abilities such as learning and memory. It has been previously shown that the histone deacetylase HDAC4 plays a critical role in memory formation in both mammals and insects although the specific mechanisms through which it acts have not yet been elucidated. HDAC4 undergoes nucleocytoplasmic shuttling and, in neurons, it is largely cytoplasmic implying it may play both nuclear and non-nuclear functions. To identify upstream regulators and downstream targets of *HDAC4*, a genetic interaction screen was performed in the fruit fly *Drosophila melanogaster*, a powerful model system to study the genetic mechanisms of neurological disease. Twenty-nine genes were found to interact with *HDAC4* suggesting they are part of the same molecular pathway. Functional network analysis revealed that many of the genes could be grouped into three biological categories comprising transcriptional factors, SUMOylation machinery enzymes and cytoskeletal regulators/interactors. Within the latter, *Ankyrin2* was selected for further analysis as it is implicated in synaptic stability and in human intellectual disability. In addition HDAC4 harbours a conserved ankyrin binding domain. Immunohistochemical analyses showed widespread distribution of Ankyrin2 throughout the adult brain and coincident distribution with HDAC4 was observed in the axons of the mushroom body, a key structure for memory formation in flies. Both *HDAC4* and *Ankyrin2* were also found to regulate mushroom body development. RNAi-mediated depletion of *Ankyrin2* in the adult brain impaired long-term memory in the courtship suppression assay, a model of associative memory and preliminary evidence of a physical association between HDAC4 and Ankyrin2 was also demonstrated. The genes identified in the screen provide new avenues for investigation of the mechanisms through which *HDAC4* regulates memory formation and preliminary analyses suggest that interaction with the cytoskeletal adaptor Ankyrin2 may involve remodelling of the actin/spectrin cytoskeleton, phenomenon that underlies memory related processes like synaptic plasticity and neuronal excitability.

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This was the year of the Summer Olympic Games and I could not help the feeling of participating to a sort of Olympic Games myself… as a PhD student. In particular, this sport event corresponded exactly with my thesis writing and made me realise that academic life and sport training share similarities. As in sport training, the good days are rare. Most of the time, it is hard, it takes a long time, things go wrong, better and then wrong again and in order to be successful at either, your commitment must be to the process, not to the final prize. However, with dedication, passion, patience and a good dose of optimism the final goal would gradually approach.

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ABBREVIATIONS

°C	Degree Celsius
AIS	Axon initial segment
Ank1	Ankyrin1
Ank2	Ankyrin2
Ank3	Ankyrin3
ANK-B	Ankyrin B
ANK-G	Ankyrin G
ANK-R	Ankyrin R
Arc1	Activity-regulated cytoskeleton associated protein 1
Att	Arginine tolerance test
Aβ	Amyloid-beta
BDSC	Bloomington Drosophila Stock Centre
bp	Base pair
Ca^{++}	Calcium
CaMK	Calcium/calmodulin-dependent kinase
cAMP	Cyclic adenosine monophosphate
cDNA	Complementary DNA
CI	Courtship index
CIP	Calf intestinal alkaline phosphatase
Cm	Centimeters
CRE	cAMP response element
CrebB	cAMP response element binding protein B
CS	Canton special
Cy	Curly
DNA	Deoxyribonucleic acid
DroID	Drosophila interactions database
dsRNA	Double stranded RNA
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
EGTA	Ethylene glycol tetraacetic acid
Elav	Embryonic lethal abnormal visual system

FasII	Fasciclin II
FLIM	Flurescence lifetime imaging microscope
FPKM	Fragments per kilobase of transcript per million mapped
FRET	Fluorescence resonance energy transfer
GFP	Green fluorescent protein
GMR	Glass multimer reporter
GST	Glutathione S-transferase
H^+	Hydrogen
HAT	Histone acetyltransferase
HCl	Hydrochloric acid
HDAC	Histone deacetylase
HDAC4	Histone deacetylase 4
HEK293	Human embryonic kidney 293 cells
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
INTACT	Isolation of nuclei tagged in specific cell types
IPTG	Isopropyl-β-D-thiogalactoside
K^+	Potassium
KCl	Potassium chloride
kDa	Kilodalton
L	Litre
LI	Learning index
LoxP	Locus of X-over P1
LTM	Long-term memory
M	Molar
mA	Milliampere
MAPK	Mitogen-activated protein kinase
Mef2	Myocyte enhancer factor 2
mg	Milligram
MgCl2	Magnesium chloride
MI	Memory index
ml	Millilitre
mm	Millimeters
mM	Millimolar
mRNA	Messenger RNA

Na^+	Sodium
NES	Nuclar export signal
ng	Nanogram
NLS	Nuclear localisation signal
nm	Nanometers
NMDARs	N-Methyl-D-Aspartic acid receptors
Nrg	Neuroglian
OE	Overexpression
PCR	Polymerase chain reaction
PKA	Protein kinase A
qPCR	Quantitative Real Time PCR
Repo	Reversed polarity
RFP	Red fluorescent protein
RNA	Ribonucleic acid
RNAi	RNA interference
RNAseq	RNA sequencing
Rpm	Revolution per minute
Sb	Stubble
SDS-PAGE	Sodium dodecyl sulphate – polyacrylamide gel electrophoresis
STM	Short-term memory
STRING	Search tool for the retrieval of interacting genes/proteins
SUMO	Small ubiquitin-like modifier
SV40	Simian virus 40
TARGET	Temporal and regional gene expression targeting
Ts	Temperature sensitive
UAS	Upstream activating sequence
Ubc9	Ubiquitin Carrier Protein 9
V	Volt
VDRC	Vienna Drosophila Resource Centre
Wt	Wild-type
µg	Microgram
µl	Microlitre
µm	Micrometer