Characterisation of the gene *deflated*, the *Drosophila melanogaster* orthologue of human Integrator subunit 7

Thesis submitted by

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Abstract

Transcription of the majority of spliceosomal RNAs, small nuclear RNAs (snRNAs), as well as all protein-coding genes is accomplished by RNA polymerase II (RNAPII). Recently, a novel protein complex called 'Integrator' was identified in human cells that associates with the C-terminal domain (CTD) of RNAPII's largest subunit. Integrator has previously been shown to be critical for the co-transcriptional 3' end processing of snRNAs, as depletion or mutation of at least two of the twelve known Integrator subunits causes 3' end misprocessed snRNAs to accumulate. Genes encoding Integrator subunits are well conserved in metazoa, including *Drosophila*, but are absent from the genomes of unicellular organisms. Previous studies of the *Drosophila* orthologue of Integrator subunit 7 (Ints7), *deflated,* associated *deflated* with cellular roles that are difficult to reconcile with a function in snRNA 3' end processing. These include pleiotropic defects in *deflated* mutants throughout the entire development indicative of functions in cell proliferation and/or cell signalling. This thesis explores the functional conservation of *deflated* as a subunit of the *Drosophila* Integrator complex and examines the possibility of *deflated* being multifunctional.

In an attempt to address the involvement of *deflated* in snRNA biogenesis as well as other cellular processes, a series of studies were conducted using *Drosophila* for experimentation. Changes in snRNA 3' end processing were analysed via quantitative real-time PCR (qPCR) in a mutant *deflated* background. In comparison to heterozygous control larvae, homozygous second instar $defl^{L}$ larvae accumulate significant amounts of 3' end misprocessed snRNA precursors and also display defects in pre-mRNA splicing of some, but not all, mRNA transcripts. This is consistent with *deflated* functioning as an Ints7 Integrator subunit and Integrator having a critical role in snRNA 3' end processing in *Drosophila*.

A functional involvement of *deflated* as a subunit of the *Drosophila* Integrator complex was further explored using the yeast two-hybrid system (Y2H). Deflated was found to bind to two of the twelve known Integrator subunits in *Drosophila* as well as to the DSS1 protein that initiated discovery of the Integrator complex (in human cells) but does not play a role in snRNA biogenesis. To explore the possibility that *deflated* is multifunctional, as indicated by previous findings in *Drosophila*, a commercial Y2H

screen was commissioned to identify non-Integrator binding partners of Deflated. Two novel physical interactors of Deflated were identified and confirmed in this study via pairwise Y2H assays. The first was the Mcm2 subunit of the MCM2-7 replicative helicase and the second the dynein light chain Dlc90F of cytoplasmic dynein. These observations support a multifunctional role of *deflated*.

In the final set of experiments, Deflated localization studies during *Drosophila* development were undertaken using a custom made anti-Deflated antibody. Immunofluorescence confocal microscopy revealed that Deflated is ubiquitously expressed in the nuclei of early *Drosophila* embryos but becomes more and more restricted in its tissue distribution with ongoing development. Anti-Deflated staining first follows sites of rapid cell proliferation and is only seen in the developing CNS and epithelia of mainly ectodermal origin from gastrulation until late larval stages. In adults, Deflated's localization was analysed in the male reproductive system, where it strongly stained primary spermatocytes, and in female ovaries, where it was found to stain all egg chamber follicle cells until mid-oogenesis. Later in oogenesis, anti-Deflated staining becomes restricted mainly to the anterior follicle cell population and a small proportion of follicle cells at the posterior pole. Using cDNA rescued homozygous $defl^{L}$ females to analyse egg chamber development in a mutant background, defects in the posterior follicle cell epithelium became apparent after mid-oogenesis. These data establish a clear link between the epithelial specific expression of Deflated and a function in epithelial organisation. An additional finding of the anti-Deflated staining was co-localization of Deflated with γ-tubulin to centrosomes in follicle cells. This localization may well reflect the identified Y2H physical interaction between Deflated and Dlc90F.

Together, these data suggest that *deflated* encodes a multifunctional protein that is required for correct snRNA 3' end formation as well as other cellular processes. The findings of this study indicate possible roles of Deflated at centrosomes, in epithelial organisation and, in association with Mcm2, in either DNA replication or transcription of protein-coding genes. Further research of *deflated* and other Integrator subunits will be required to reveal the full extent of functions beyond Integrator's role in snRNA 3' end processing and why Integrator complex subunits are restricted to only metazoan species.

Table of contents

List of Figures

List of Tables

List of Abbreviations

3D	three dimensional
aa	amino acids
AMG	anterior midline glia
bp	base pairs
cDNA	DNA synthesized from a mRNA template
CNS	central nervous system
CO_2	carbon dioxide
Co-IP	co-immunoprecipitation
CPSF	cleavage and polyadentylation specific factor
CTD	C-terminal domain
C-terminus (terminal)	(at the) carboxy-terminal end of a polypeptide chain
DGRC	*Drosophila* Genomic Resource Center
dH_2O	distilled water
DSE	distal sequence element
ECL	enhanced chemi-luminescence
ERK	extracellular signal-regulated kinase
GMC	ganglion mother cell
IPC	inner proliferation center of the optic lobe
kb	kilo base pairs
kDa	kilo Dalton
MAPK	mitogen-activated protein kinase
MET	mesenchymal-epithelial transition
MG	midline glia
MIP	maximum intensity projection
mRNA	messenger RNA
N-terminus (terminal)	(at the) amino-terminal end of a polypeptide chain
OD_{600}	optical density at 600nm (measured in a spectrophotometer)
OPC	outer proliferation center of the optic lobe
PCR	polymerase chain reaction
PMG	posterior midline glia
PNE	precephalic neuroectoderm
PNS	periperpheral nervous system
pre-mRNA⇒	pre messenger RNA
PSE	promoter specific element
qPCR	quantitative real-time PCR
RNA	ribonucleic acid
RNAPII	DNA dependent RNA polymerase II
rpm	revolutions per minute
RTK	receptor tyrosine kinase
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
Ser^2 (5 or 7)	serine number 2, 5 or 7 of RNAPII CTD's heptapeptide repeats
snRNA	small nuclear RNA
snRNP	small nuclear ribonucleoprotein
SPG	subperineurial glia
UAS	upstream activation sequence
UTR	untranslated region
VNC	ventral nerve cord
VNE	ventral neuroectoderm
ZA	zonula adherens