Genetic and cellular analysis of the novel cell proliferation regulator, *deflated*, in *Drosophila*

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Statement of contribution of others

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Abstract

The regulation of cell proliferation needs to be coordinated with cell growth and differentiation regulation in multicellular organisms for development to occur properly and tumourogenesis to be avoided. Cell proliferation is regulated through positive and negative signals that influence progression through the cell cycle. The cell cycle is regulated by cyclin dependent kinases that are controlled by the binding of cyclins, whose protein levels oscillate throughout the cell cycle. It is extremely important that progression through the cell cycle is regulated correctly, that mitosis only occurs once DNA replication is completed, and that DNA replication only begins once chromosomes have segregated into daughter cells. While there is great understanding about how the different phases of the cell cycle are controlled, it is still largely unknown how the phases are coordinately regulated, and how the cell cycle is regulated externally during development. An understanding of how cell proliferation is coordinated with cell growth and differentiation requires an examination of how the cell integrates various and sometimes competing signals with the core cell cycle machinery to effect cell proliferation in the right developmental context.

This study describes the identification of *deflated* as a novel cell proliferation regulator. Taking a genetic and cell biological approach, the role that *deflated* plays in regulating cell proliferation was examined. *deflated* alleles were generated by P-element mobilisation and P-element induced male recombination. The characterisation of four different alleles and the expression pattern of *deflated* mRNA suggested a role for *deflated* in regulating cell proliferation and cell signalling. These findings were further supported by genetic interactions observed between *deflated* and cell proliferation

regulators and cell signalling components. Examination of *deflated* function in Ras signalling demonstrated that *deflated* may be a negative regulator of Ras signalling. The findings that DEFLATED::GFP protein localised to nuclei and mitotic spindles in embryos and to the apical surface of wing imaginal discs, further support a role for *deflated* in cell proliferation and cell signalling.

When taken together, these data suggest both a direct and an indirect role for *deflated* in regulating cell proliferation. It appears that DEFLATEDmay be involved in regulating re-replication and thus link DNA synthesis completion with M-phase entry. DEFLATED may also regulate spindle function and formation, possibly by forming a complex with Ran and clathrin, two proteins that are predicted to bind DEFLATED and have roles in spindle function. *deflated* may also regulate cell proliferation indirectly through involvement in Ras signalling, the Ran pathway, or through endocytosis. While these are many roles to ascribe to DEFLATED it may be able to carry them all or a subset out by functioning as an adaptor protein at the intersection of different cellular pathways.

This initial analysis is the first to characterise *deflated* function in any model system and it is clear that *deflated* is a novel regulator of cell proliferation. Further elucidation of its function will enable an understanding of how sensing and signalling pathways communicate with one another to ensure that cell proliferation occurs correctly. In particular, the study of *deflated* will enable further understanding on how Ras signalling regulates cell proliferation and how M-phase and the mitotic spindle are regulated.

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List of Abbreviations

3-D	three dimensional
a.a.	amino acid
APC/C	anaphase promoting complex/cyclosome
BCIP	5-bromo-4-chloro-3-indolyl-phosphate
BMP	bone morphogen protein
BrdU	bromodeoxyuridine
bp	base pairs
cdk	cyclin dependent kinase
CNS	central nervous system
dNTP	deoxyribonucleoside triphosphate
DIG	digoxigenin
Dpp	decapentaplegic
EGFR	epidermal growth factor receptor
Gbb	glass bottom boat
GFP	green fluorescent protein
GTP	guanidine triphosphate
kb	kilobase pairs
KSR	kinase suppressor of Raf
l	litre
MAPK	mitogen activated protein kinase
MEK	MAPK/Erk kinase
MPF	mitotis promoting factor
NBT	nitroblue tetrazolium chloride
NES	nuclear export signal
NLS	nuclear localisation signal
OD_{600nm}	optical density at 600nm
ORC	origin replication complex
PCNA	proliferating cell nuclear antigen
PCR	polymerase chain reaction
PNS	peripheral nervous system
RNR2	ribonucleoside diphosphate reductase
rpm	revolutions per minute
RTK	receptor tyrosine kinase
Wg	wingless