The versatile transcription factor Oct-1: A crucial protein in embryonic development and A key component of the stress cellular response.

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Contents

Abbreviations

Aa:	amino acids
BrdU:	bromodeoxyuridine
CDC:	cell division cycle
CDK:	cyclin dependent kinase
CDKI:	cyclin dependent kinase inhibitor
CFSE:	carboxyfluorescein diacetate succimidyl ester
ChIP:	chromatin immunoprecipitation
CRM1:	chromosome region maintenance
DNA:	desoxyribonucleic acid
EGF:	epidermal growth factor
ERK:	extracellular-signal regulated kinase
ES cells:	embryonic stem cells
ExE:	extra-embryonic ectoderm
F,f allele:	flox allele
FACS:	fluorescence-activated cell sorting
FGF:	fibroblast growth factor
GADD45:	growth arrest DNA damage
GDP:	guanosine diphosphate
GTP:	guanosine triphosphate
Gy:	gray
H&E:	hematoxilin and eosin
ICM:	inner cell mass
KO:	knock-out

MAPK:	mitogen activated protein kinase
MEF:	mouse embryonic fibroblast
NEBD:	nuclear envelope break-down
PCR:	polymerase chain reaction
PGC:	primordial germ cells
PKB:	protein kinase B
Rb:	retinoblastoma
RNA:	ribonucleic acid
RT:	reverse transcriptase
RTK:	receptor tyrosine kinase
SH2:	sarcoma homology domain
TE:	trophectoderm
TGF-β:	transforming growth factor- β
TOR:	target of rapamycin
TS cells:	trophoblastic stem cells
UV:	ultraviolet
Wt:	wild type

Summary of the thesis

Chapter 1

Oct-1 is a transcription factor belonging to the POU family (Clerc et al. 1988) (Herr et al. 1988) (Ryan and Rosenfeld 1997). The members of this family are involved in a broad range of biological processes like transcription of housekeeping genes (Oct-1), pluripotency of embryonic stem cells (Oct-4) or development of immune system (Oct-1, Oct-2) (Spaniol et al. 1996). The transcription factor Oct-1 is ubiquitously expressed in embryonic and adult tissues, and regulates the expression of a variety of genes. Previous studies described Oct-1 to be regulated at the protein level by phosphorylation in a cell cycle dependent manner. In addition, more recently it has been shown that Oct-1 is induced in response to DNA damage and modulates the activity of genes like *GADD45* important for the cellular stress response (Segil et al. 1991) (Zhao et al. 2000) (Jin et al. 2001) (Fan et al. 2002) (Tantin et al. 2005)..

Knockout and a conditional *oct-1* knockout alleles were created in our laboratory, and used to generate Oct-1 deficient mouse embryonic fibroblast (MEFs) and embryonic stem cells (ES). We used them as a model to study the cellular response to stress in absence of Oct-1. We have found that cells lacking Oct-1 were less sensitive to stress like γ-irradiation, deprivation of glucose or amino acids but not to serum starvation or H2O2 treatment. Under glucose starvation, this effect is in part mediated by activation of the signalling protein ERK and PKB which leads to maintenance of the level of cyclin D1 protein in KO cells

comparable to the level without treatment in heterozygous or rescue cells. These proliferative signals allow to KO cells to bypass the G1 cell cycle checkpoint and to proliferate better. Under stress conditions like glucose starvation, Oct-1 regulates the cell cycle by blocking cells in G1 phase, and by controlling the expression of the cyclin dependent kinase inhibitor p27.

Chapter 1 of this thesis provides a general introduction about the regulation of cell cycle and, more particularly, about key proteins and important pathways of the progression and transition of G0/G1 and S phases. It also gives some background about pathways like PKB and ERK, or other signaling pathways like p53, which plays determinant roles in cellular responses after stress induction. The role of the transcription factor Oct-1 in cell cycle regulation in response to cellular stress is discussed in the results part.

Chapter 2

The POU transcription factors family was identified after the isolation of three mammalian transcription factors and a *Caenorhabditis elegans* developmental regulator: Pit-1, Oct-1, Oct-2 and Unc-86 (Ryan and Rosenfeld 1997). Oct-1 and Oct-2 proteins show a high degree of identity (90%) but differ in their expression pattern. Oct-1 is ubiquitously expressed, whereas Oct-2 is restricted to B cells, macrophages, hematopoietic cells, as well as cells of the central nervous system (Gerster et al. 1987) (He et al. 1989) (Staudt et al. 1988). Despite several studies, the exact role of Oct-1 and Oct-2 in immunoglobulin genes regulation and B-cell development is not well understood. In order to

investigate the functions of Oct-1 in B cells, our laboratory created a deficient mouse for Oct-1. Mice homozygous for *oct-1 (-/-)* were generated by crossing heterozygous +/- mice. Unfortunatly, these matings failed to produce any live born homozygous *oct-1 -/-* mutant, indicating that Oct-1 is essential for embryonic mouse development.

In this second project, the result part of chapter 2 will focus on the lethal phenotype observed during embryogenesis in absence of Oct-1 and gives an overview of the possible role of Oct-1 during mouse embryonic development. We showed that the embryos die around 8 dpc due to an arrest of the ectoplacental cone and giant trophoblastic cells development which occurs at 6.5dpc. This extraembryonic deletion leads to the formation of an impaired mesoderm.

In introduction the chapter 2 gives a general background about the different developmental embryonic stages in mouse and describes in more details the gastrulation and the different pathways and factors associated.