

Abstract

In *Drosophila*, the maternally localized gene *pumilio* is thought to act as a regulator of the posterior pattern in *Drosophila* embryo by binding to sites in the *hunchback* gene named Nanos Response Elements (NREs). Binding of *pumilio* to these sites represses the translation of the *hunchback* gene.

Recent work, at the University of Leeds, has identified a cDNA clone from the moss *Physcomitrella patens* cDNA library, that by sequence analysis showed to have strong homology with *Drosophila pumilio* gene. In this work, this cDNA clone (pPum 3a) was used as a probe to screen *Physcomitrella patens* genomic library constructed in bacteriophage vector λ -GEM 12(provided by Dr. M. Leech at John Innes Center). Southern blot has identified a fragment which was cloned at the Sst I site of the vector pBluescript SK+. The ca. 5.5kb genomic clone pGPum S2.2 , had homology with the 3'-region of the cDNA clone. Another similar work by Miss Irene Wier at the University of Leeds has identified another ca. 4 kb genomic clone (pGPums E461)(cloned at the Eco RI site of pBluescriptSK+) that has homology with both the 3'- region and 5'- region of the cDNA clone (pPum 3a). This clone was sequenced at this work. The first approach in sequencing was by creating nested deletions within the cloned ca. 4 kb fragment. Sequence and computer analysis of this clone suggest two readings to the protein coding region of the *Physcomitrella pumilio* gene and that the isolated clone might not be the whole *Physcomitrella pumilio* gene. They also suggest that there could be a family of *pumilio* related genes within the moss *Physcomitrella patens*.

Contents:

Abstract	I
Contents	II
Tables and Figures	VI
Abbreviations	VIII
Acknowledgment	X

Chapter one: Introduction

1.1 Animal development	3
1.2 Early development in <i>Drosophila melanogaster</i>	3
1.2.1 The anteroposterior axis	4
1.2.2 The anterior morphogenetic gradient	5
1.2.3 The posterior morphogenetic gradient	7
1.3 Conserved functions in animal development	11
1.3.1 Early development in <i>Caenorhabditis elegans</i>	11
1.3.2 Conservation of functions in vertebrates	14
1.3.3 <i>Pumilio</i> homologues in plant and fungi	15
1.4 Plant development	17
1.4.1 Early development in <i>Arabidopsis thaliana</i>	18
1.4.2 Fucus: embryogenesis in isolation	22
1.4.3 <i>Physcomitrella patens</i>	23

Chapter two: Materials & Methods:

2.1 Materials	26
2.2 Media and solutions	29
2.3 DNA extraction	32
2.3.1 Large scale plasmid DNA extraction	32
2.3.2 Small scale plasmid DNA extraction	33
2.3.3 Extraction of phage Lambda DNA	33
2.4 DNA purification and concentration	34
2.4.1 Phenol chloroform extraction	34
2.4.2 Ethanol Precipitation	34
2.4.3 CsCl/Ethidium bromide density gradient centrifugation	34
2.5 Agarose gel electrophoresis	35
2.6 Recovery of DNA from agarose gels	35
2.7 Determination of DNA size and concentration	36
2.8 Restriction endonuclease Digestion of DNA	36
2.9 Ligation of DNA	37
2.10 Preparation of competent cells for transformation with plasmid DNA	38
2.11 Storage of competent cells	38
2.12 Transformation of competent cells with plasmid DNA	39
2.12.1 CaCl ₂ transformation	39
2.12.2 Blue / white colony screen	39
2.13 Radiolabeling of DNA	40

2.13.1 Radiolabeling of DNA using oligolabelling	40
2.13.2 5'-end labeling of primers	40
2.14 Purification of radiolabelled DNA	41
2.15 Scintillation counting of probes	41
2.16 Southern DNA transfer	42
2.16.1 Binding of the DNA to the membrane	42
2.16.2 Prehybridisation	43
2.16.3 Hybridization	43
2.16.4 Washing	43
2.16.5 Autoradiography	44
2.16.6 Developing Autoradiograph	44
2.17 Plaque Hybridisation	45
2.18 Exonuclease III/ mung bean nuclease deletions	45
2.19 DNA sequence determination	47
2.20 Computer analysis	49
<u>Chapter three: Characterization of pumilio genomic clones</u>	
3.1 Screening of moss genomic library	51
3.2 Isolation of three genomic clones	52
3.3 Analysis of the isolated clones	57
3.4 Subcloning the genomic digests into pBluescript SK+	61

Chapter four: Analysis of genomic subclones

4.1 Restriction analysis of clones pGPumS2.2 and pGPum E461	66
4.2 Sequence analysis of clone pGPum E461	67
4.3 Computer analysis of sequence data	77

Chapter five: Discussion

5.1 Pumilio-like sequences from <i>Physcomitrella patens</i>	88
5.1.1 Homologies between clones	88
5.1.2 Coding potential of pGPum E461	91
5.1.3 Database homology searches with pGPum E461 DNA sequences	96
5.1.4 A proposed DNA sequence	99
5.1.5 Homologies between pumilio like proteins	104
5.2 Future priorities	104
5.2.1 Further Characterization of moss genes	107
5.2.2 What is the biological role of pumilio-like gene?	108
5.2.3 Biochemical function of the pumilio like protein	110
5.3 Conclusions	112
<u>References</u>	114

ABBREVIATIONS

A	:	Adenine
Amp	:	Ampicillin
bp	:	base pairs
C	:	Cytosine
CCC	:	Covalent Closed Circular
cpm	:	counts per minute
ddNTP	:	2'-3' dideoxynucleoside triphosphate
DNA	:	deoxyribonucleic acid
cDNA	:	complementary DNA
dNTP	:	2'-deoxy nucleoside triphosphate
EDTA	:	ethylene dinitrilotetra acetic acid, sodium salt
EtBr	:	3,8,-diamino-5-ethyl-6-phenylphen-anthridium bromide (ethidium bromide)
G	:	Guanine
IPTG	:	Isopropyl- β -D-thiogalacto pyranoside
kb	:	kilo base pair
min.	:	minute
M_r	:	relative molecular mass
OD	:	Optical Density
O/N	:	Over night
RNA	:	ribonucleic acid
mRNA	:	messenger RNA
rpm	:	revolution per minute
SDS	:	sodium dodecyl sulphate
T	:	thymine
Tet	:	tetracycline
Tris	:	2-amino-2-(hydroxymethyl)-1,3-propanediol
UV	:	ultra violet
v/v	:	volume(ml) per volume(ml)
w/v	:	weight(g) per volume(ml)
X-gal	:	5-bromo-4-chloro-3- β -D-galactopyranoside
Δ	:	deletion

Amino Acids

C	Cys	Cysteine
A	Ala	Alanine
G	Gly	Glycine
N	Asn	Asparagine
S	Ser	Serine
F	Phe	Phenylalanine
P	Pro	Proline
L	Leu	Leucine
Q	Gln	Glutamine
V	Val	Valine
D	Asp	Aspartic Acid Aspartate
E	Glu	Glutamic acid Glutamine
I	Ile	Isoleucine
W	Trp	Tryptophan
R	Arg	Arginine
M	Met	Methionine
Y	Tyr	Tyrosine
H	His	Histidine
K	Lys	Lysine
T	Thr	Threonine