Figures

Fig. 1. Effects of PSK-α on non-embryogenic cell proliferation in the presence of different concentrations of 2,4-D. Non-embryogenic cells were incubated in liquid medium containing PSK-α at the indicated concentrations with various concentrations of 2,4-D (A, 4.5 x 10^{-6} M; B, 4.5 x 10^{-7} M). The cell density was adjusted to 0.2 ml PCV/l and cells were counted on the 14th day of culture. Data are the means of three replicates ± SD (the bar is not shown where it was too small to be displayed).

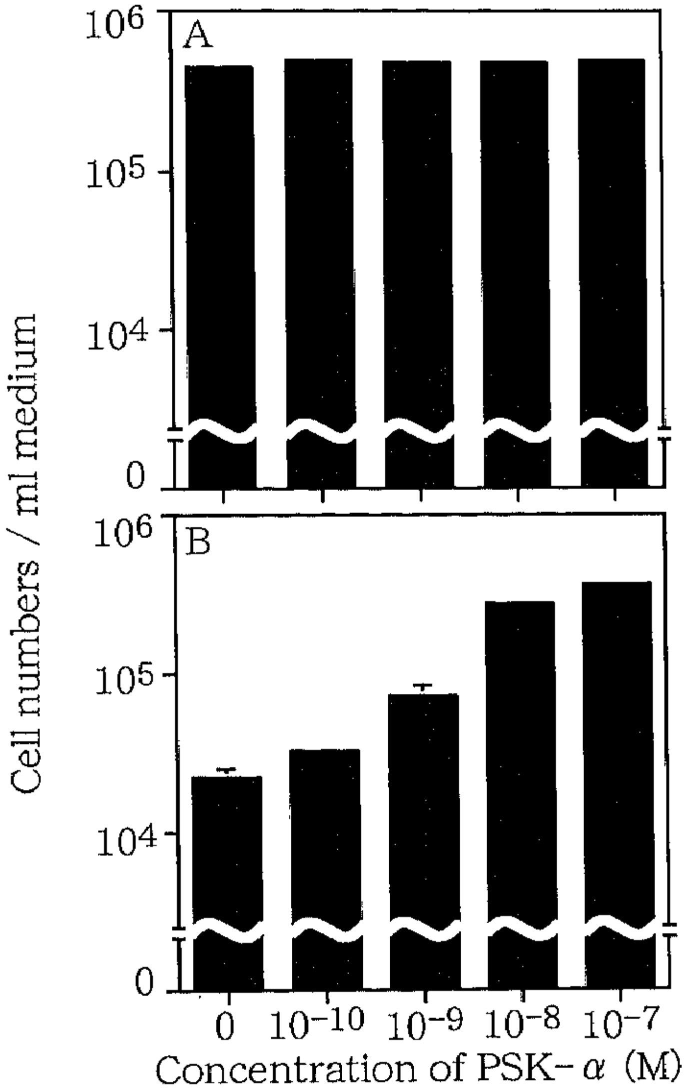

A
B
10^6
10^5
10^4
0
0 10^-10 10^-9 10^-8 10^-7
Concentration of PSK-α (M)
Cell numbers / ml medium

Fig. 2. Effects of PSK-α on non-embryogenic cell proliferation in the presence of different concentrations of IAA (A) and NAA (B). Non-embryogenic cells were incubated in liquid medium containing PSK-α at the indicated concentrations with various concentrations of IAA (a, 4.5 x 10^{-6} M; b, 4.5 x 10^{-7} M; c, 4.5 x 10^{-8} M) and NAA (a, 4.5 x 10^{-6} M; b, 4.5 x 10^{-7} M; c, 4.5 x 10^{-8} M). The cell density was adjusted to 0.2 ml PCV/l, and cells were counted on the 14th day of culture. Data are the means of three replicates ± SD (the bar is not shown where it was too small to be displayed).

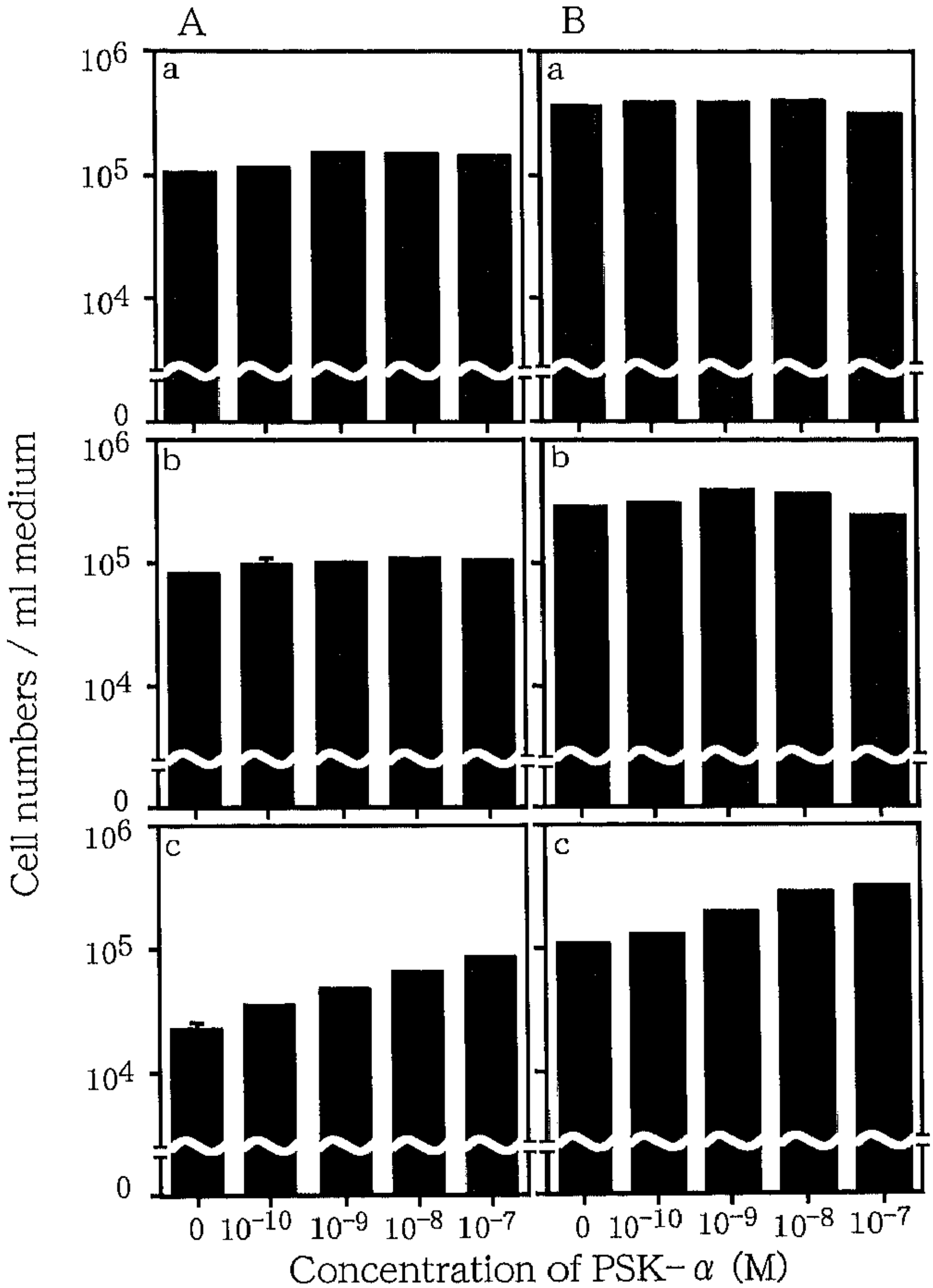

A
B
a
b
c
10^6
10^5
10^4
0
Cell numbers / ml medium
0 10^{-10} 10^{-9} 10^{-8} 10^{-7}
Concentration of PSK-α (M)

Fig. 3. PSK-α accumulation in CM derived from non-embryogenic cell culture in the presence of high (H) or low (L) concentrations of auxin (2,4-D, IAA, and NAA). Non-embryogenic cells were suspended at 0.2 ml PCV/l and cultured in liquid medium that contained 4.5 x 10^{-6} M (H) or 4.5 x 10^{-7} M (L) of auxin. The PSK-α concentration in the medium was determined by competitive ELISA. Data are the means of duplicates ± SD (the bar is not shown where it was too small to be displayed).

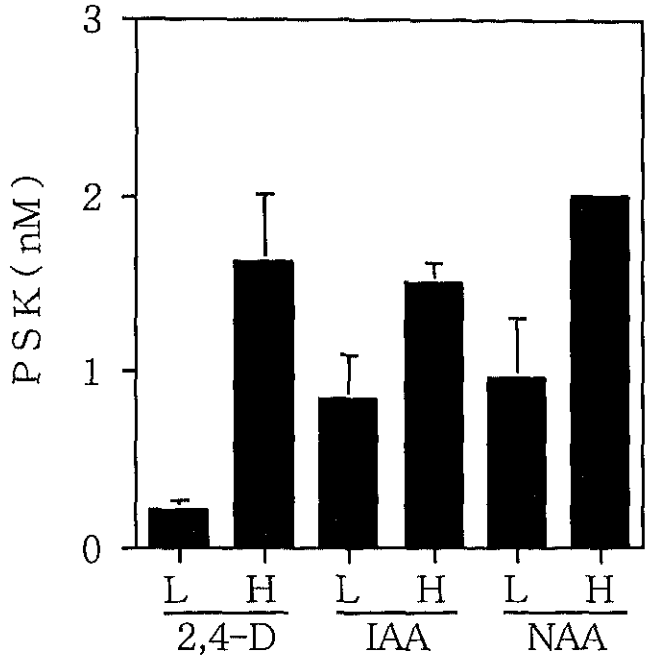
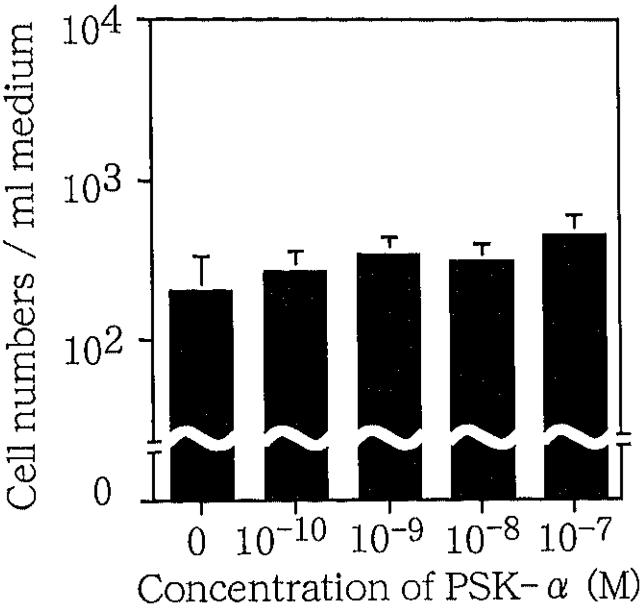

3
2
1
0
P S K (nM)
L
H
L
H
L
H
2,4-D
IAA
NAA

Fig. 4. Effects of PSK-α on non-embryogenic cell proliferation in the absence of 2,4-D. Non-embryogenic cells were precultured in medium without 2,4-D for 14 days and then incubated in liquid medium containing PSK-α at the indicated concentrations without 2,4-D. The cell density was adjusted to 0.2 ml PCV/l, and cells were counted on the 14th day of culture. Data are the means of three replicates ±SD.



Cell numbers / ml medium
10^4
10^3
10^2
0
0
10^-10
10^-9
10^-8
10^-7
Concentration of PSK-α (M)

Fig. 5. Flow cytometry analysis of non-embryogenic cells after release from propyzamide blocking in M phase. A time course of the accumulation of cells in G1- or G2/M- phases of the cell cycle was examined in cell suspensions cultured in the presence of a low concentration (4.5×10^{-7} M) of 2,4-D without (A and D) or with (B and E) PSK- α (1×10^{-7} M), and in the presence of a high concentration (4.5×10^{-6} M) of 2,4-D without PSK- α (C and F). Closed and open symbols indicate the percentage of the cells accumulated in the G1 phase or G2/M phases, respectively. Each experiment was performed in duplicate, and the results of each experiment are shown separately (1st and 2nd experiment).

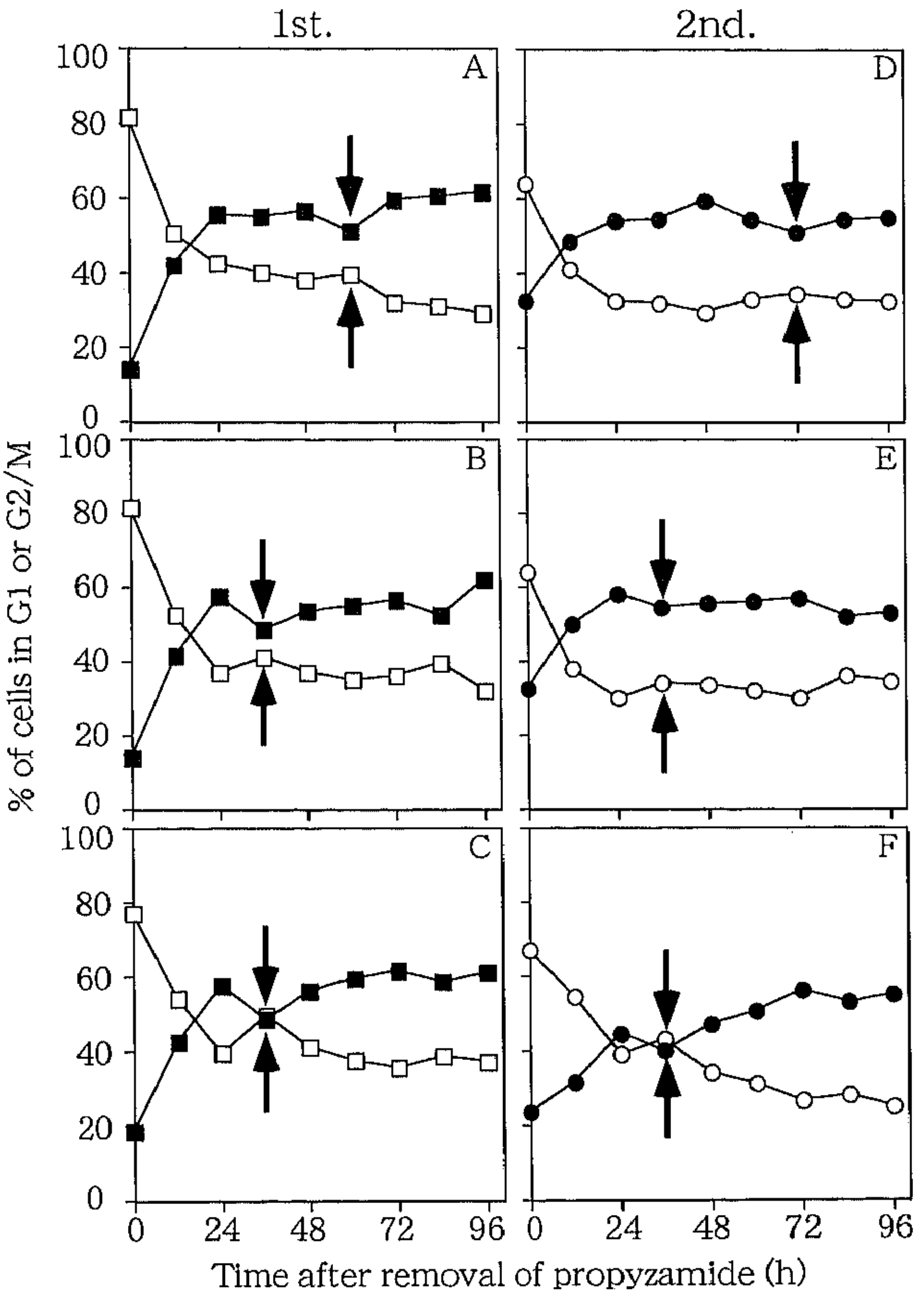

1st.
2nd.
A
B
C
D
E
F
100
80
60
40
20
0
0
24
48
72
96
% of cells in G1 or G2/M
Time after removal of propyzamide (h)

Fig. 6. Effects of PSK-α on cell division in non-embryogenic cells after release of propyzamide blocking. Cell numbers were counted each day for four days. The initial cell density was adjusted to 0.2 ml PCV/l. Each experiment includes three replicates, and all experiments were performed in duplicate. Data are the means with standard deviation (where the bar is not shown, it was too small to be displayed). ■, low-concentration (4.5 x 10^{-7} M) of 2,4-D without PSK-α; ●, low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK-α (1 x 10^{-7} M); ▲, high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK-α.

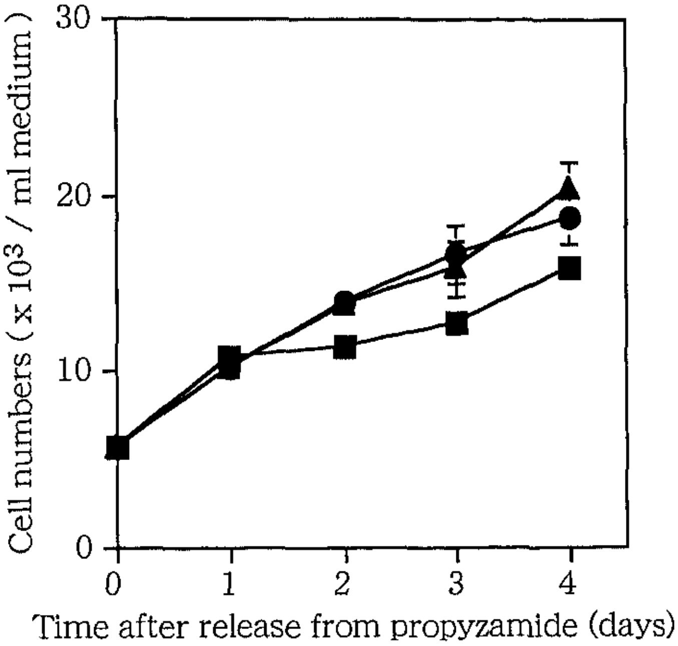

Cell numbers (x 10^3 / ml medium)
30
20
10
0
0
1
2
3
4
Time after release from propyzamide (days)

Fig. 7. Nucleotide sequence of the carrot *CycD2* cDNA and deduced amino acid sequence. The initiation codon of the open reading frame is indicated by a double underline, and the LxCxE motif is shown in boldface letters. The cyclin box is shown in black, and a putative poly A signal sequence is underlined.

1 ACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGTGGAGACACATATAAAATTACATAT 60

61 GCTCACACAACACAGCAACACACATTTATAAATGTATATATAAAGCTCACTTCTTTGCTA 120

121 CAGTCTATCTATCCCAAACTTCATTTCATCACTTTACAGCCATGCTTCTTGATTTCTTTA 180

181 TATCAAGTTCTCTAATTTCTTATTCATTTTAGATGAGATTCACTAGGGTTTACACAGAGG 240

M R F T R V Y T E V

241 TTTTCAATATGACAGACCATAGCCTCCTCTGCACAGAAACCAATAACTTGTGTTTTGATG 300

F N M T D H S **L L C T E** T N N L C F D D

301 ATCTTGAGGCTAGAGATGATCAGGACCCGAGAATAGATTGTGAAAATGTGGTGGGTAATG 360

L E A R D D Q D P R I D C E N V V G N E

361 AATCAGAAGCTTTGATTTGTGCCGTCCCATTACAGAGAGATGAAGATTTTGTGTTTGTGT 420

S E A L I C A V P L Q R D E D F V F V F

421 TTGAgAGCAAGGTGAATTTTTGCCCAGAGGTGATTTTCTCCACAGATAGAAGTGGTGAGC 480

E S K V N F C P E V I F S T D R S G E L

481 TTGATTTGTGTGTCAGAAAAGAGGCCCTTGATTGGATTTATAAGGCTCATGCTCATTACA 540

D L C V R K E A L D W I Y K A H A H Y N

541 ATTTTGGAGCATTGAGTGTTTGCTTAGCAGTGAATTATTTGGATCGCTTTCTTTCTCTTT 600

F G A L S V C L A V N Y L D R F L S L Y

601 ATGAATTGCCGAGTGGAAAAAAGTGGACTGTCCAATTGTTAGCTGTTGCCTGTTTATCAC 660

E L P S G K K W T V Q L L A V A C L S L

661 TGGCAGCAAAAATGGAGGAGGTTAATGTGCCACTAACTGTTGATTTACAGGTGGCTGATC 720

A A K M E E V N V P L T V D L Q V A D P

721 CTAAGTTCGTGTTCGAGGCGAAAACGATTAAAAGAATGGAGCTTTTGGTGTTGAGCACCT 780

K F V F E A K T I K R M E L L V L S T L

781 TGAAATGGAGAATGCAAGCCTGCACCCCTTGTTCATTCATAGATTACTTCCTTCGAAAAA 840

K W R M Q A C T P C S F I D Y F L R K I

841 TCAACAATGCTGATGCGCTTCCATCGGGGTCTCTGATCGATAGGTCGATTCAGTTCATTT 900

N N A D A L P S G S L I D R S I Q F I L

901 TGAAAACGATGAAAGGTATTGATTTTCTGGAATTCAGGCCCTCAGAAATTTCAGCAGCTG 960

K T M K G I D F L E F R P S E I S A A V

961 TGGCAATTTGTGTAACAAGAGAAGCACAAACACTAGACATTAATAAGGCAATGTCTAATA 1020

A I C V T R E A Q T L D I N K A M S N I

1021 TCATACCTGTTGAAAAGGATAGAGTATTCAAGTGTATTGAAATGATTCAAGATCTGACAT 1080

I P V E K D R V F K C I E M I Q D L T L

1081 TGGTTACTGAGACTAGTAATGTAGCTAGTGGTAGAACAAGAGCACAAGTGCCACAAAGTC 1140

V T E T S N V A S G R T R A Q V P Q S P

1141 CTGTTGGGGTGTTGGATGCTGCATGCTTGAGCTATAAGAGTGATGAGAGAACAGTTGGGT 1200

V G V L D A A C L S Y K S D E R T V G S

1201 CATGTCCTAATTCTTCTTTACATACTGAGACTAGTCCACACACTaAAAGGAGGAAGCTGA 1260

C P N S S L H T E T S P H T K R R K L I

1261 TTGAGATCATGAAATGTGGATTTTACTCTATCACTCAGTTTCAGATTTTTAGTGAATGGG 1320

E I M K C G F Y S I T Q F Q I F S E W E

1321 AGTTTTGGTGTGGCTGCCTTTGCAAGCGACCaCCTTTATGATAGAGGAAAAATAATATAT 1380

F W C G C L C K R P P L * *

1381 AAATATAAAAATATGTAGAGAGAgAGAgAGGGGAgAgAGAATcTTGTGCCTTGAGCATGA 1440

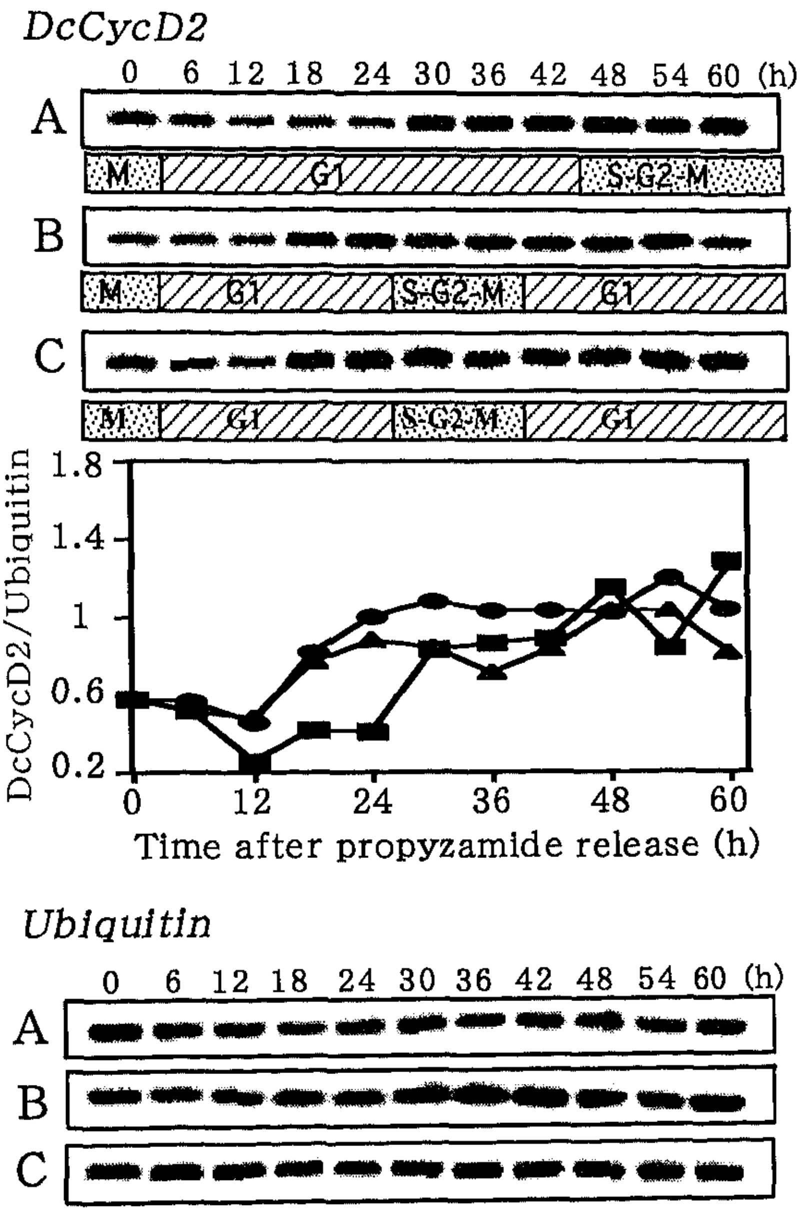
1441 ATATAGTTGTGACCAGCTGTTAATAAAATGTCATTTTAACAGTAAAACTTAGTGTGGGGG 1500

1501 AAGTGGCCGGGCCAGGAGTAaAgAGGTTTTTGCTATTTCAGTTGTTTATTAATTATTATT 1560

1561 ACTACATACAGAATATATTCTTCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 1620

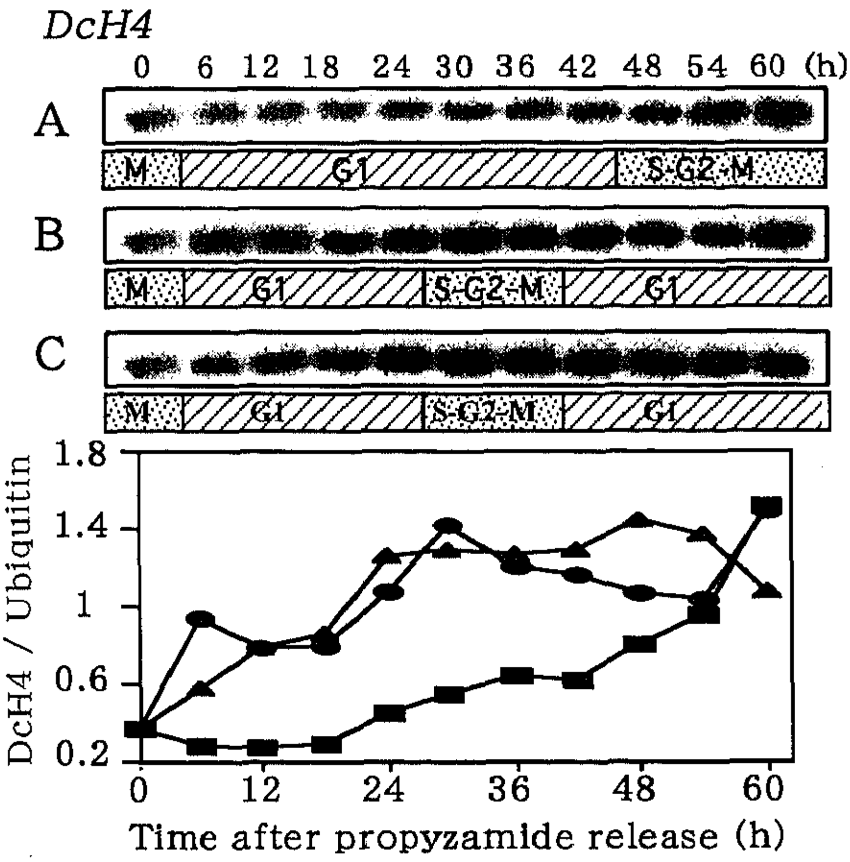
1621 CAAAAAAAAAAAAAAA 1636

Fig. 8. RT-PCR for the *DcCycD2* and ubiquitin mRNA in non-embryogenic cells after release from propyzamide blocking. Culture conditions after propyzamide release were as follows: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK-α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. Quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments. Rectangular bars below each expression data show the cell cycle expected from FCM analysis of Fig. 5.



DcCycD2
0 6 12 18 24 30 36 42 48 54 60 (h)
A
M
G1
S-G2-M
B
M
G1
S-G2-M
G1
C
M
G1
S-G2-M
G1
DcCycD2/Ubiquitin
1.8
1.4
1
0.6
0.2
0
12
24
36
48
60
Time after propyzamide release (h)
Ubiquitin
0 6 12 18 24 30 36 42 48 54 60 (h)
A
B
C

Fig. 9. RT-PCR for the *DcH4* mRNA in non-embryogenic cells after release from propyzamide blocking. Culture conditions after propyzamide release were as follows: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK-α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. Quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments. Rectangular bars below each expression data show the cell cycle expected from FCM analysis of Fig. 5.



DcH4
0 6 12 18 24 30 36 42 48 54 60 (h)
A
M
G1
S-G2-M
B
M
G1
S-G2-M
G1
C
M
G1
S-G2-M
G1
1.8
1.4
1
0.6
0.2
DcH4 / Ubiquitin
0
12
24
36
48
60
Time after propyzamide release (h)

Fig. 10. RT-PCR for the *DcCycB1;1* mRNA in non-embryogenic cells after release from propyzamide blocking. Culture conditions after propyzamide release were as follows: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK-α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. Quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments. Rectangular bars below each expression data show the cell cycle expected from FCM analysis of Fig. 5.

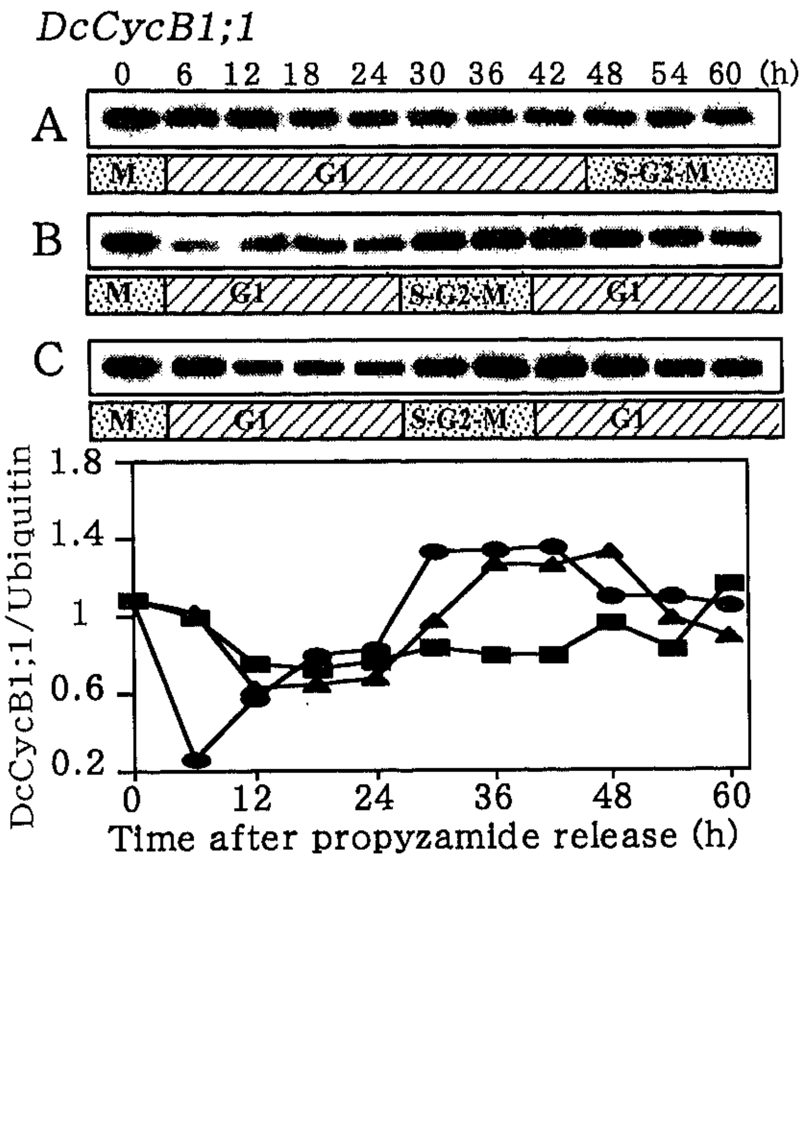

DcCycB1;1
0 6 12 18 24 30 36 42 48 54 60 (h)
A
M
G1
S-G2-M
B
M
G1
S-G2-M
G1
C
M
G1
S-G2-M
G1
DcCycB1;1/Ubiquitin
1.8
1.4
1
0.6
0.2
0
12
24
36
48
60
Time after propyzamide release (h)

Fig. 11. Flow cytometry analysis of non-embryogenic cells after addition of propyzamide with aphidicolin blocking release. A time course of the accumulation of cells in the G2/M phase of the cell cycle was examined in a cell suspension cultured in the presence of a low concentration (4.5×10^{-7} M) of 2,4-D without (■ and □) or with (● and ○) PSK- α (1×10^{-7} M), and in the presence of a high concentration (4.5×10^{-6} M) of 2,4-D without PSK- α (▲ and △). Each experiment was performed in duplicate, and the results of each experiment are shown separately (1st and 2nd experiment).

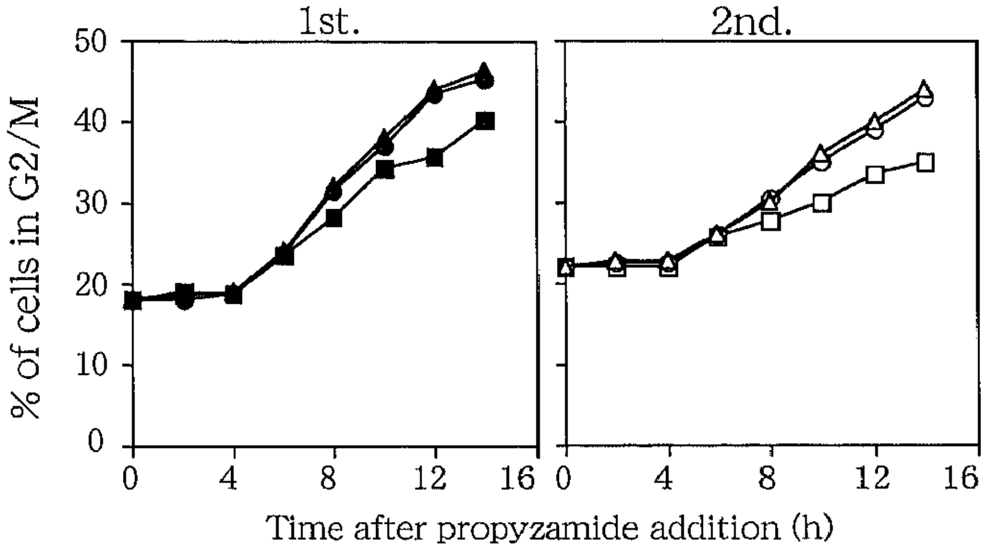

1st.
2nd.
% of cells in G2/M
Time after propyzamide addition (h)
0
10
20
30
40
50
0
4
8
12
16

Fig. 12. The nuclear DNA C-value of quiescent non-embryogenic cells. Non-embryogenic cells cultured in phytohormone-free MS medium for seven days were used in FCM analysis.

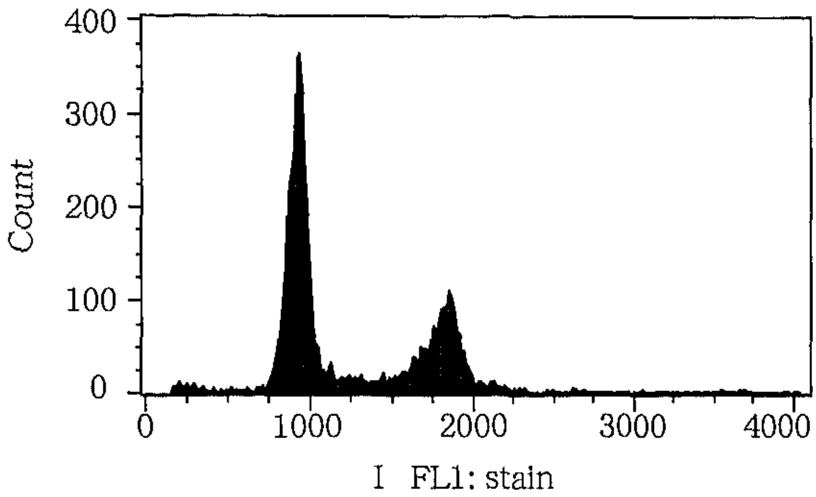
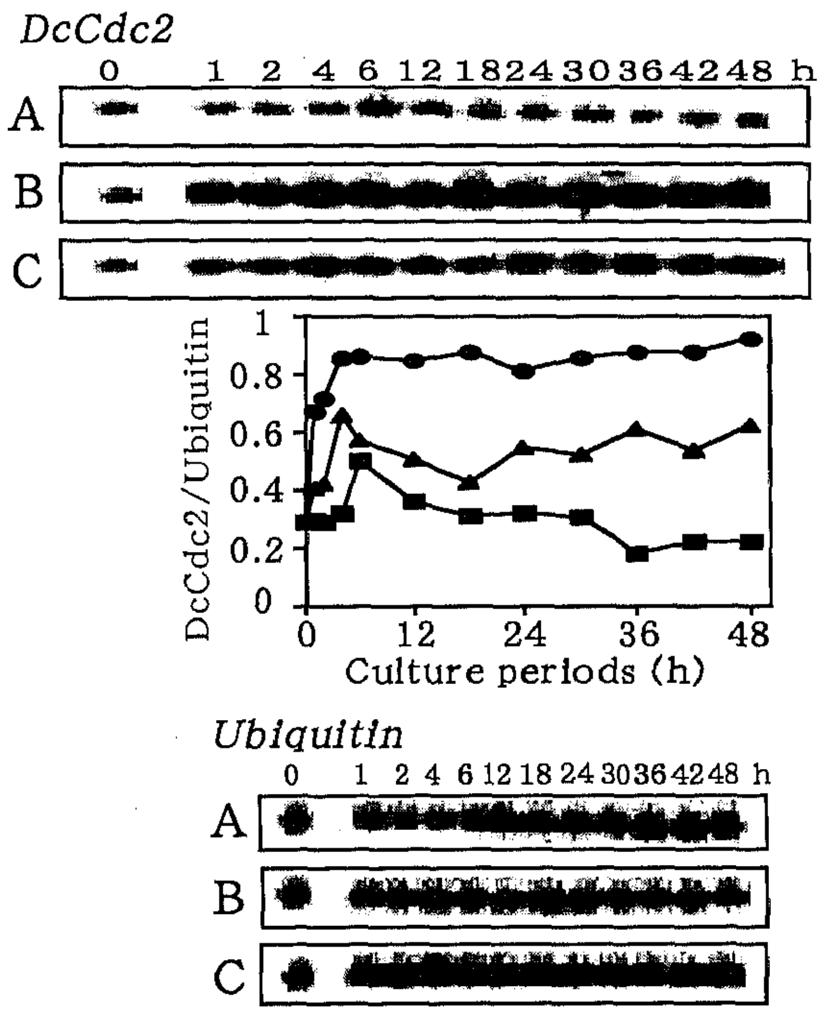

400
300
200
100
0
Count
0
1000
2000
3000
4000
I FL1: stain

Fig. 13. RT-PCR for *DcCdc2* and ubiquitin mRNA in non-embryogenic cells after re-entry into the cell cycle from the quiescent state. Quiescent non-embryogenic cells were cultured under the following conditions: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK- α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. The quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments.



DcCdc2
0 1 2 4 6 12 18 24 30 36 42 48 h
A
B
C
1
0.8
0.6
0.4
0.2
0
DcCdc2/Ubiquitin
0 12 24 36 48
Culture periods (h)
Ubiquitin
0 1 2 4 6 12 18 24 30 36 42 48 h
A
B
C

Fig. 14. RT-PCR for *DcCycD2* mRNA in non-embryogenic cells after re-entry into the cell cycle from the quiescent state. Quiescent non-embryogenic cells were cultured under the following conditions: (A, ■), low concentration (4.5×10^{-7} M) of 2,4-D without PSK- α; (B, ●), low concentration (4.5×10^{-7} M) of 2,4-D with PSK- α (1×10^{-7} M); (C, ▲), high concentration (4.5×10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. The quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments.

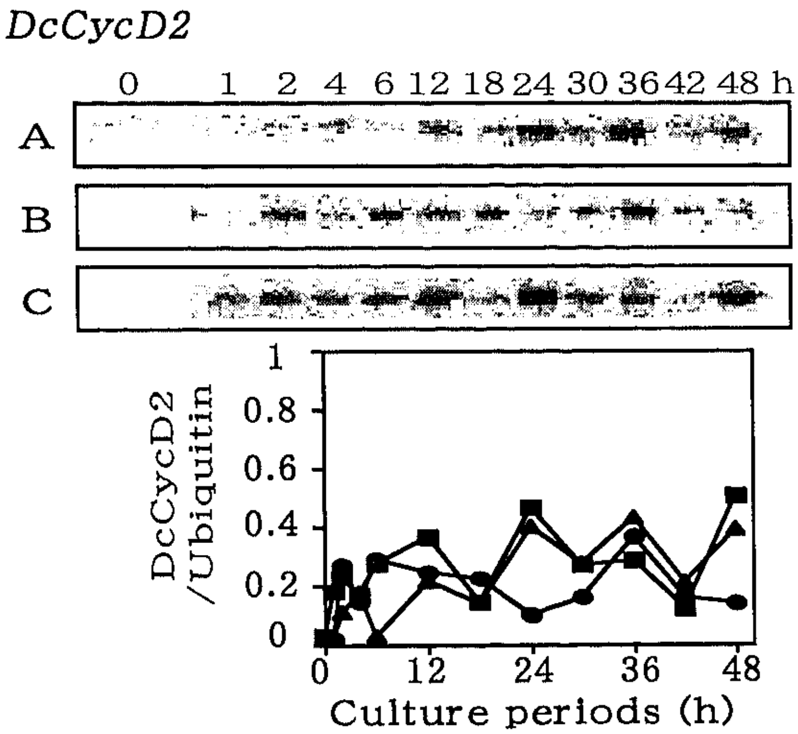

DcCycD2
0 1 2 4 6 12 18 24 30 36 42 48 h
A
B
C
DcCycD2 /Ubiquitin
1
0.8
0.6
0.4
0.2
0
0 12 24 36 48
Culture periods (h)

Fig. 15. RT-PCR for *DcH4* mRNA in non-embryogenic cells after re-entry into the cell cycle from the quiescent state. Quiescent non-embryogenic cells were cultured under the following conditions: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK- α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. The quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments.

DcH4

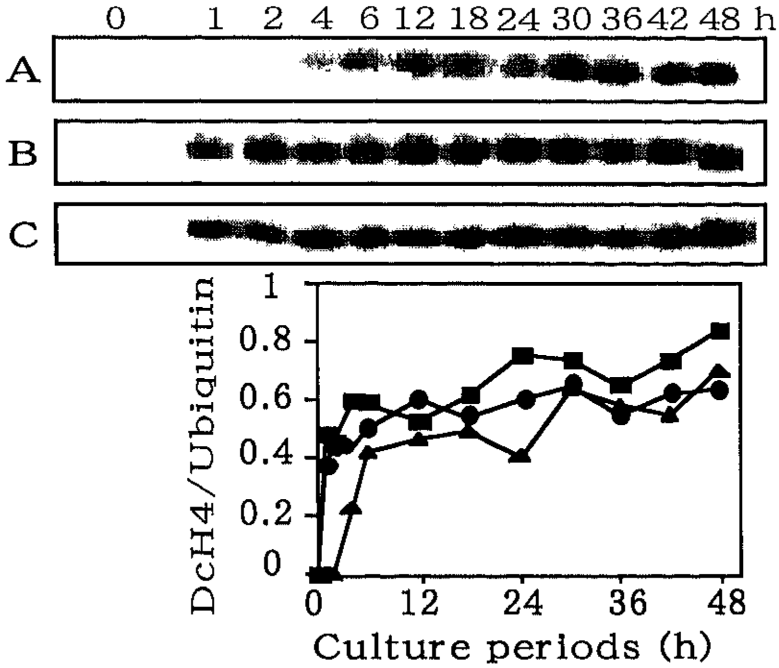
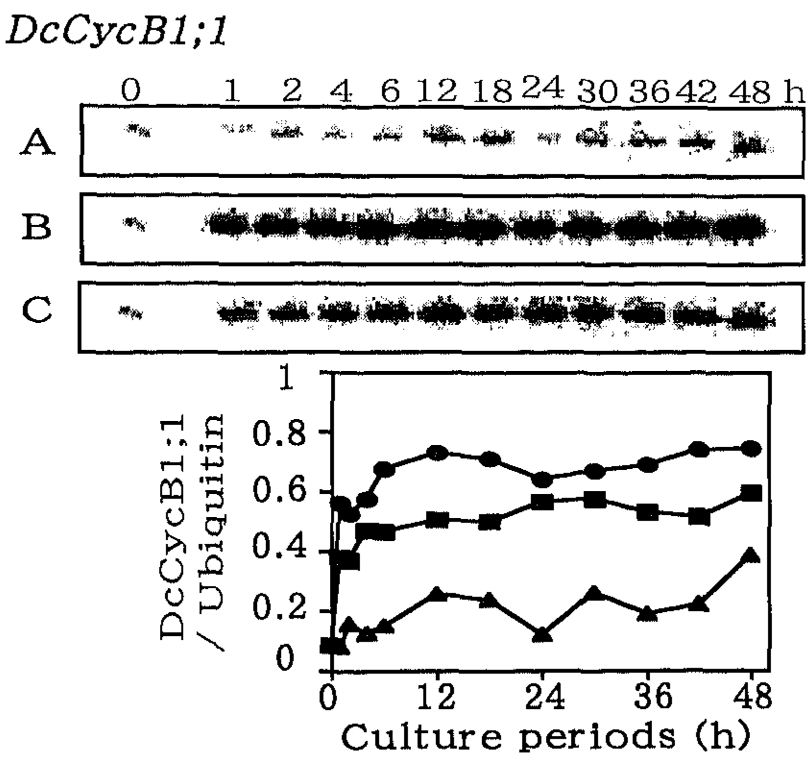
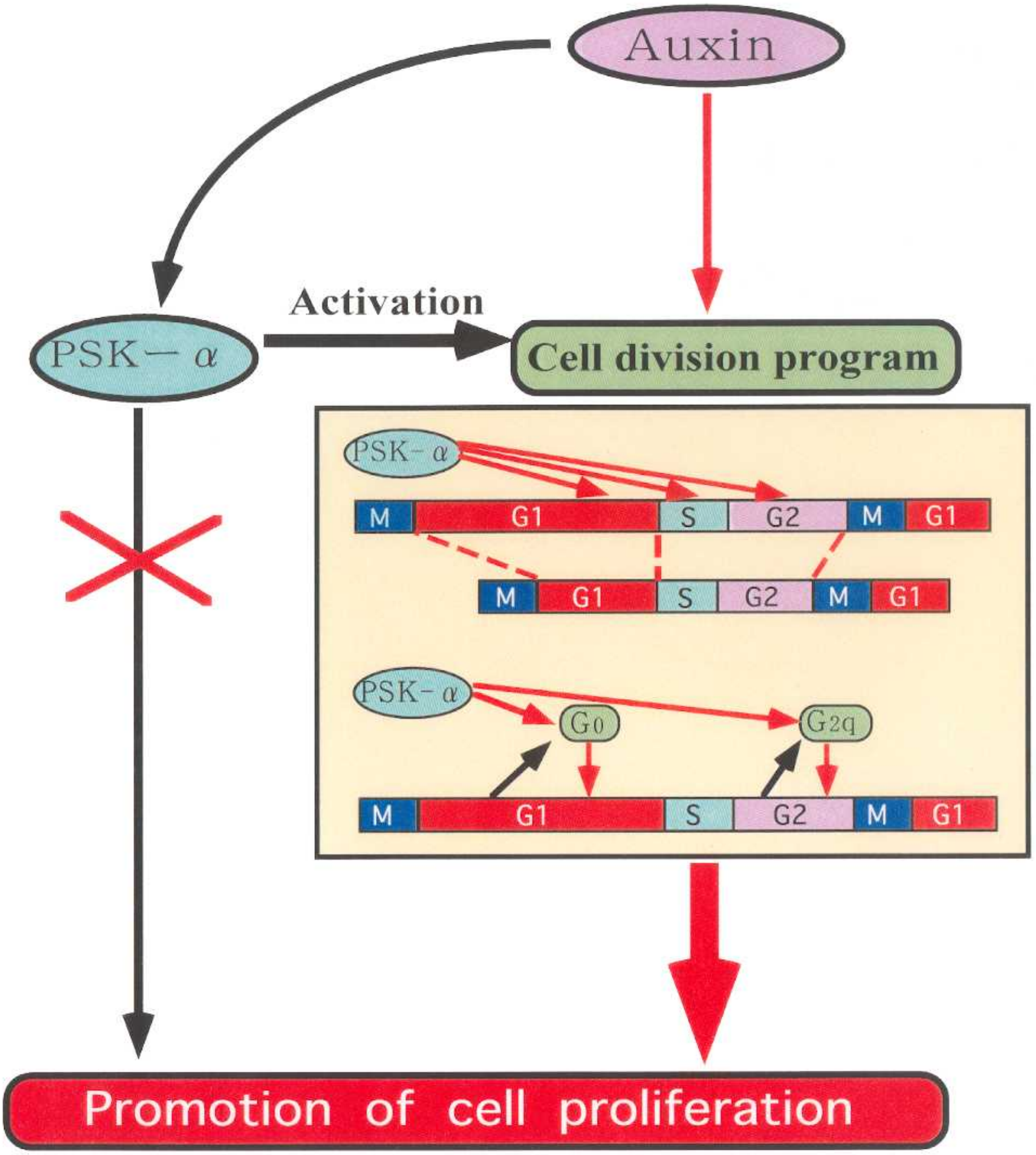

0 1 2 4 6 12 18 24 30 36 42 48 h
A
B
C
DcH4/Ubiquitin
1
0.8
0.6
0.4
0.2
0
0 12 24 36 48
Culture periods (h)

Fig. 16. RT-PCR for *DcCycB1;1* in non-embryogenic cells after re-entry into the cell cycle from the quiescent state. Quiescent non-embryogenic cells were cultured under the following conditions: (A, ■), low concentration (4.5 x 10^{-7} M) of 2,4-D without PSK- α; (B, ●), low concentration (4.5 x 10^{-7} M) of 2,4-D with PSK- α (1 x 10^{-7} M); (C, ▲), high concentration (4.5 x 10^{-6} M) of 2,4-D without PSK- α. Hybridization signals were quantified using a Fuji BAS5000 Imaging Analyzer. The quantified data were equalized by normalizing to the amount of ubiquitin signal. Each experiment was performed in duplicate and the figure shows representative experiments.



DcCycB1;1
0 1 2 4 6 12 18 24 30 36 42 48 h
A
B
C
DcCycB1;1 / Ubiquitin
1
0.8
0.6
0.4
0.2
0
0 12 24 36 48
Culture periods (h)

Fig. 17. A schematic model for the promotion of cell proliferation by PSK-α. Auxin involves in the production of PSK-α and the PSK-α stimulates the cell proliferation by activating the cell cycle induced by auxin.


Auxin
Activation
PSK－α
Cell division program
PSK- α
M
G1
S
G2
M
G1
M
G1
S
G2
M
G1
PSK- α
G0
G2q
M
G1
S
G2
M
G1
Promotion of cell proliferation