Recherche de nouvelles molécules à propriétés insecticides

Observation de départ :

Si on ajoute des champignons lyophylisés dans le milieu nutritionel de la drosophile, on observe une mortalité des larves.

100 % toxicity when added in the medium mg ml^{-1}

Amanita phalloïdes	0.1
Boletus (Xer.) *chrysenteron*	0.5
Boletus (Bol.) *radicans*	2
Clitocybe nebularis	3
Hygrophorus chrysodon	3
Clitopilus prunulus	4
Hygropphorus niveus	5
Lepista inversa	5
Megacollybia platyphylla	5
Tricholoma sulphureum	5
Lepista nuda	5

Antifeeding properties of mushroom on *Spodoptera littoralis*

	Average
Macrolepiota rahcoides	***-0.81***
Amanita Phalloides	***- 0.74***
Clitopilus prunulus	***- 0.71***
Agaricus romagnesii	***- 0.69***
Clytocybe nebularis	***- 0.65***

Toxicity extraction with water

Species	Toxicity (%)
Amanita phalloides	99.9
Xerocomus chrysenteron	94
Xerocomus subtomentosus	89
Lepista nuda	95
Xerocomus badius	90
Boletus speciocus	90

Insecticidal properties originate from hydrophilic compounds

Effect of dialysis on toxicity

Species	% Proteins	% Toxicity
Amanita phalloides	100	63
Xerocomus chrysenteron	84	98
Xerocomus subtomentosus	99	99
Lepista nuda	94	99
Boletus speciocus	100	88

Macromolecules are reponsible for insecticidal activity

La toxicité est thermosensible

Conclusion :
des protéines sont responsables de l’activité insecticide

Effect of proteolytic treatment

Species	remaining toxicity (%)
Amanita phalloides	89
Xerocomus chrysenteron	96
Xerocomus subtomentosus	197
Lepista nuda	65
Xerocomus badius	61

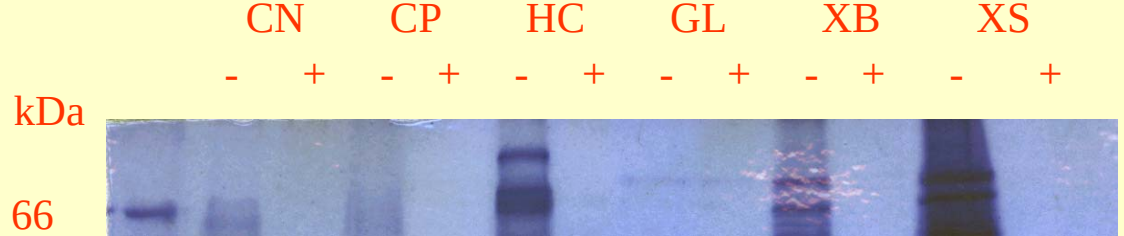
Toxicity is resistant to proteolysis

Hypotheses:

1) It exists protease inhibitor in mushroom extracts

Proteolysis is efficient in mushroom extracts, but some proteins are resistant

Effect of protease treatment on mushroom proteins



Antiproteases did not show any insecticidal activity

Proteolysis does not affect le lectin activity (hemaglutination activity)

Effect of proteolytic treatment on lectin

Species	Lectin

Lectins as good candidates for insecticidal properties

- Lectins are resistant to proteolysis as insecticidal properties.

X. chrysenteron lectin

Purification using stroma chromatography allowed to purified a lectin which recognizes galactose

Lectins from *X. chrysenteron* was then purified with lactosyl sepharose.

β mercaptoethanol	+		-	
	1.5 µg	15 µg	1.5 µg	15 µg

XCL have insecticidal and nematicidal activities but represents only a part of the insecticidal property of *X. chrysenteron*

Toxicity comparison of crude extract and purified lectin from *X. chrysenteron*

Cloning of the *Xerocomus chrysenteron* lectin

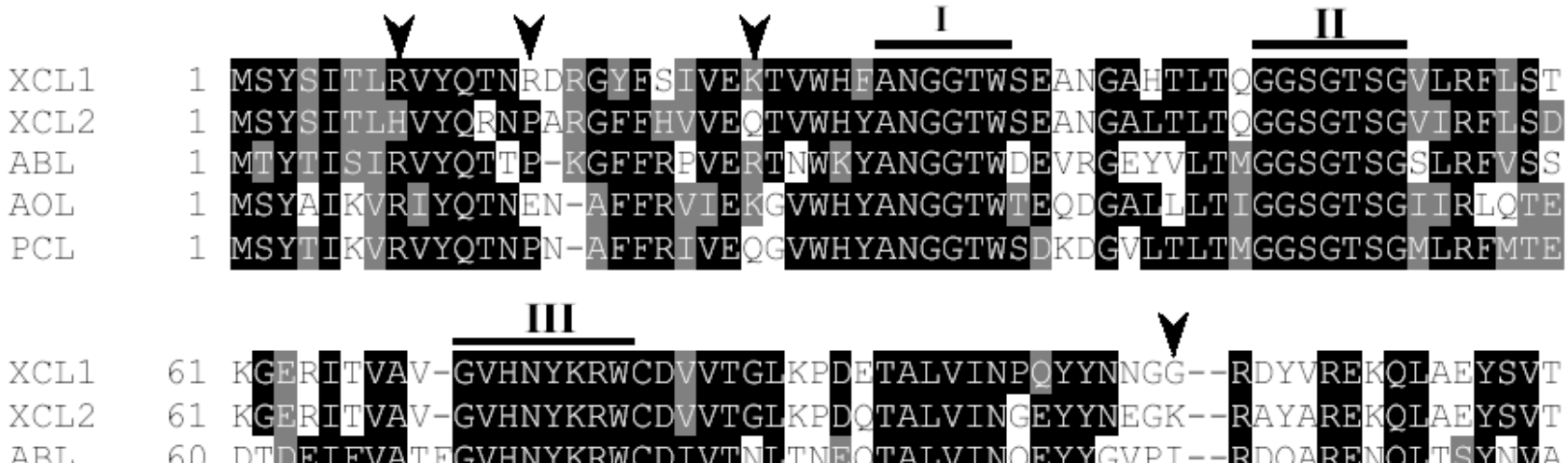
Library of cDNA

✓ Immunological screening:

- Rabbit antibodies directed against the native protein do not recognize the denatured proteins.

- Denatured protein was not immunogenic

At least two genes encode *X. chrysenteron* lectin

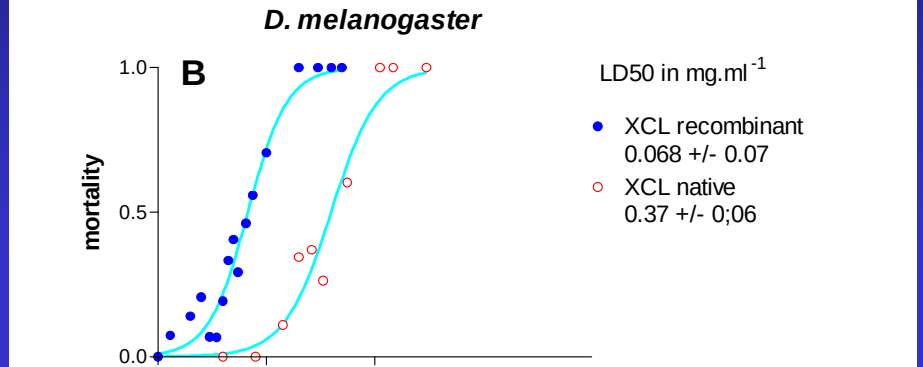


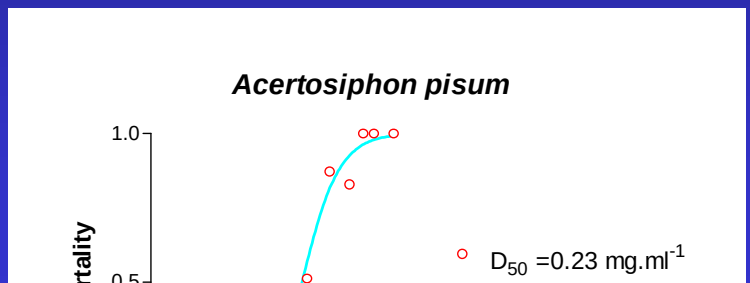
Production of recombinant XCL in *E. coli*

Production in *E. coli* (pTag His LXC)
purification on Ni bound chromatography
Concentration : 1.3 mg ml^{-1}

	Sample 1				Sample 2	recombinant protein
β mercaptoethanol	+		-		+	+
	1.5 µg	15 µg	1.5 µg	15 µg	10 µg	20 µg

Toxicité de la lectine recombinante XCL1

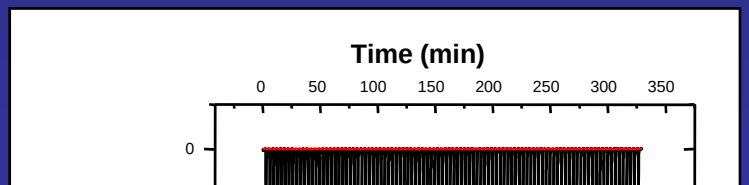




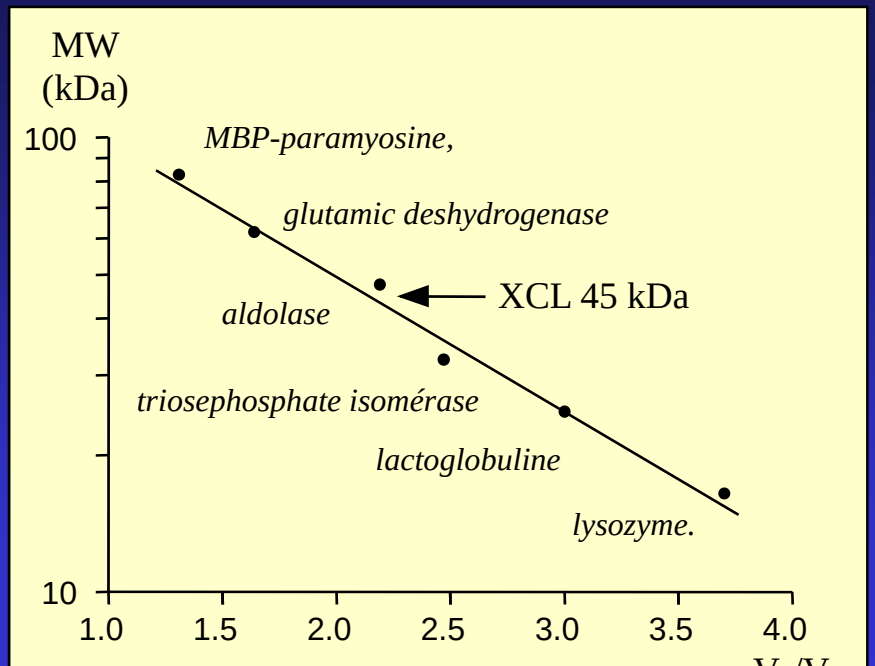
Sugar – XCL interaction

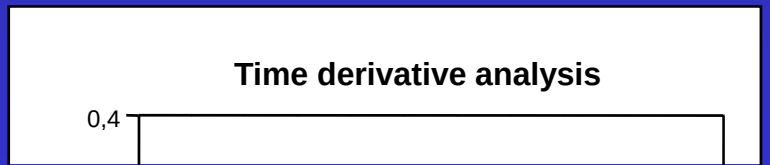
Red blood cell agglutination by XCL

- was inhibited when galactose, lactose and N-acetyl-galactosamine was added to the system
- but no effect was seen with glucose, fucose, fructose, sorbitol, mannose and sucrose

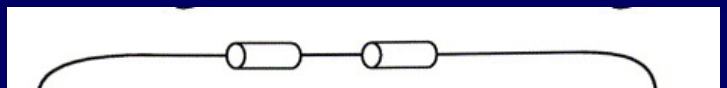


Estimation of molecular weight and polymerisation

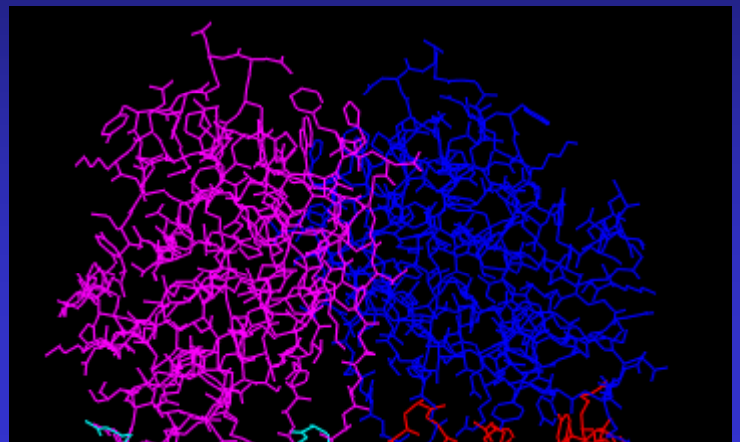


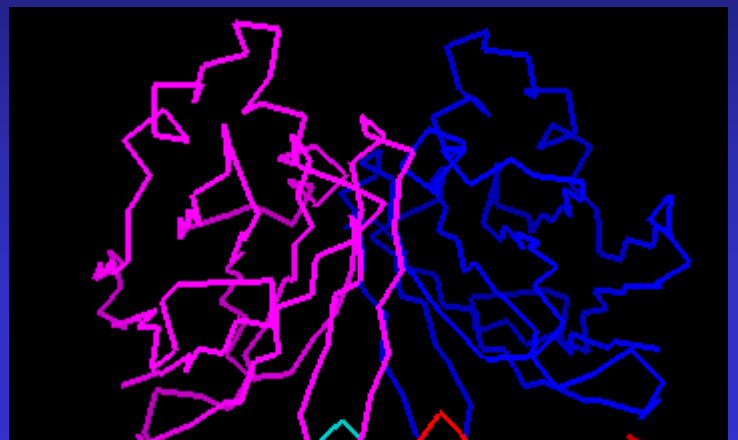


XCL presente la même structure que les actinoporines

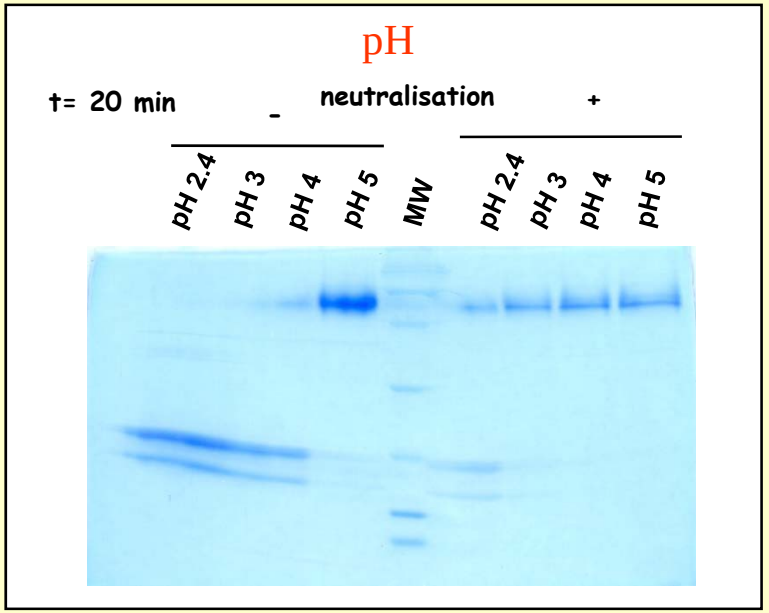


La tétramérisation forme une cavité de 1000 Å^3

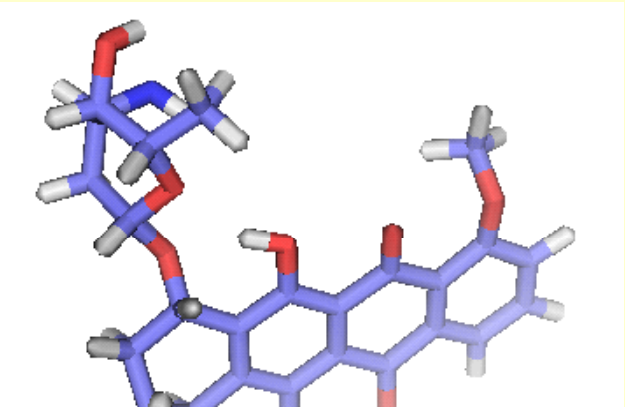


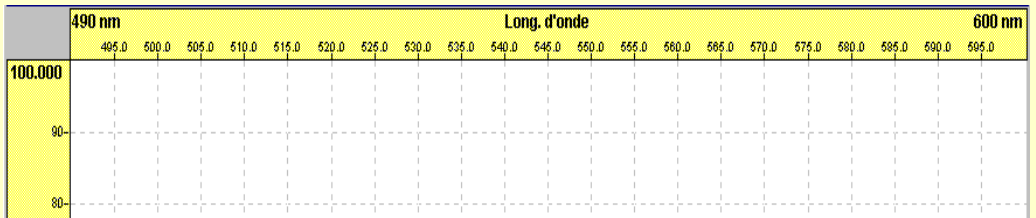


Denaturation de XCL

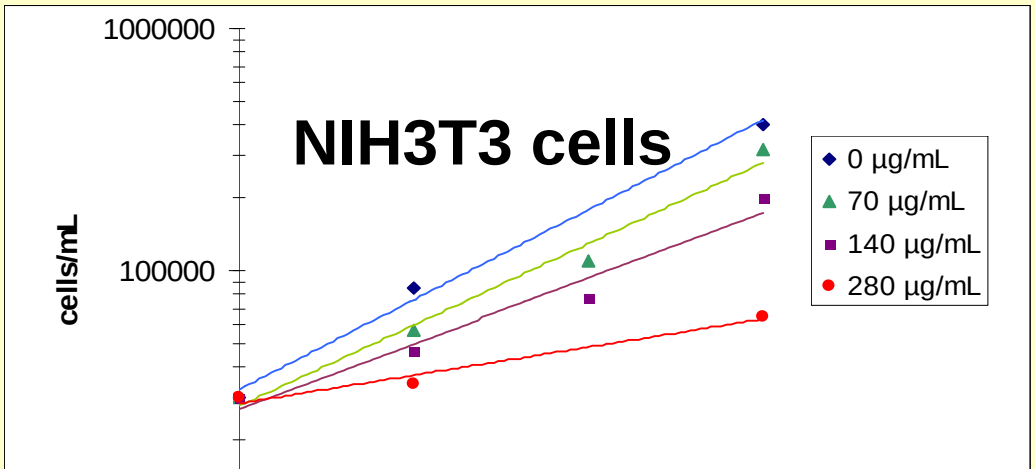


Doxorubicine loading in XCL





Effect of XCL1 on cell proliferation

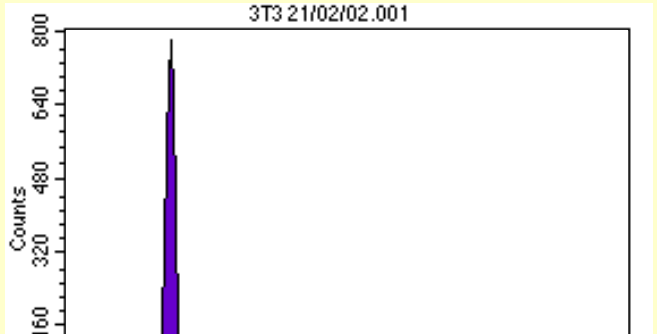


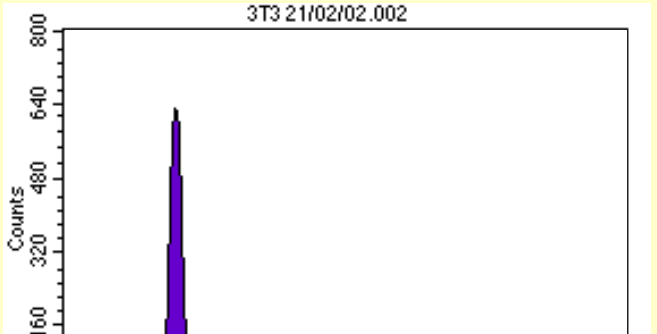
XCL1 does not block cells at a specific stage of the cell cycle and does not induce apoptose

CONTROL CELLS

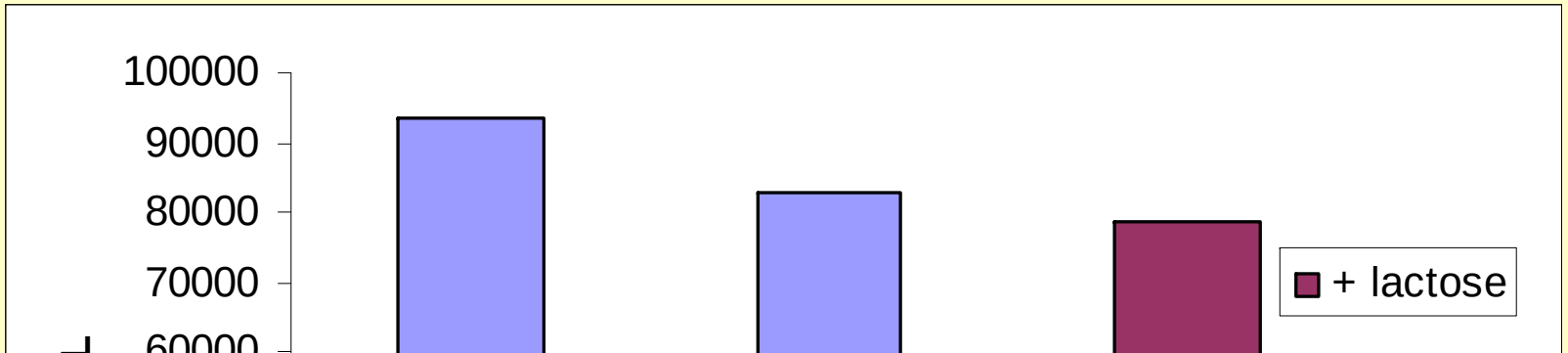
TRAITED CELLS

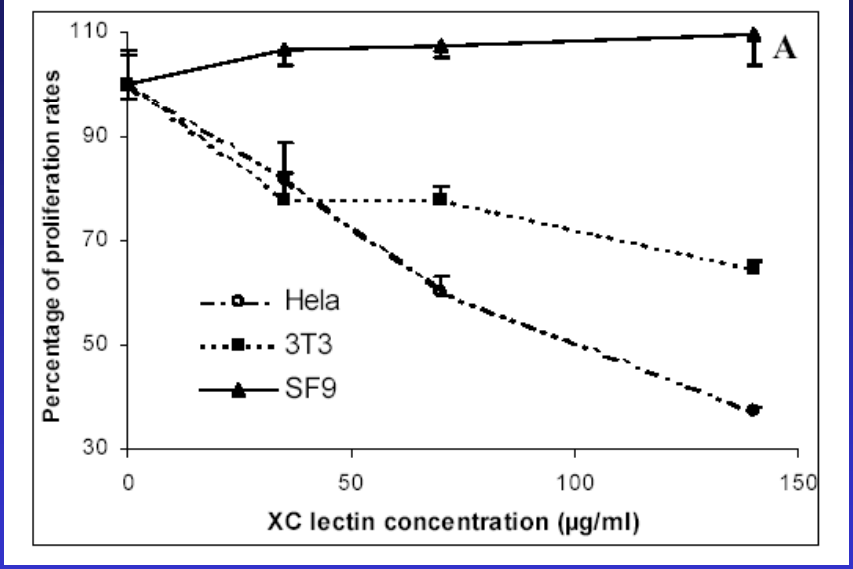
NIH-3T3 cells



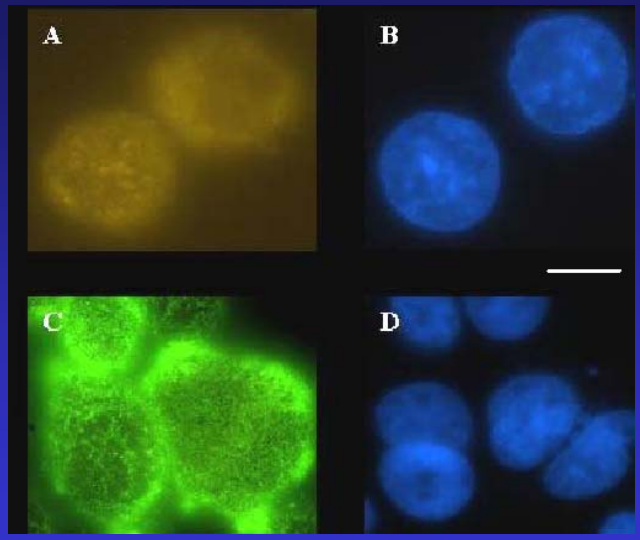


Lactose inhibits the antiproliferative effect of XCL1 on NIH3T3 cells





Effect of XCL on cell proliferation



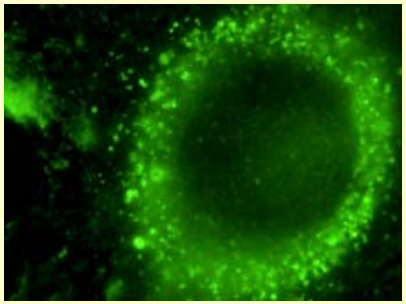
Cell surface binding of XCL

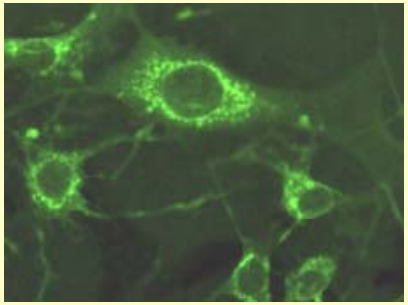
Disruption of cell-substrate adhesion seems to be the main factor affecting cell growth inhibition.

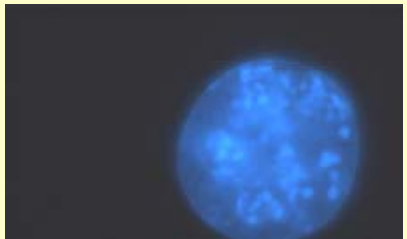
i) No antiproliferative effect was observed on the SF9 cell line, which does not require to be attached to grow.

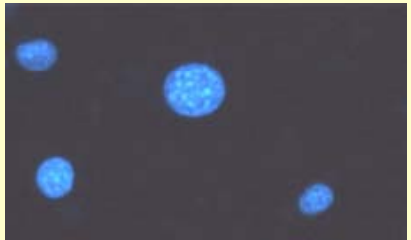
ii) XCL was shown to affect the adherence of cells following their suspension by trypsin treatment.

iii) XCL was localized on the cell surface where it would act as a coating agent.









Endocytosis of XCL in different cell lines.

Localisation de XCL1

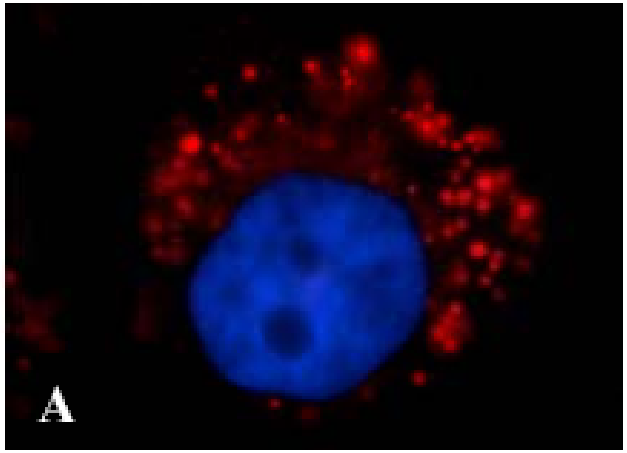
- Colocalisation avec le catepsin D : endosomes tardifs, lysosomes
- Pas de colocalisation avec la lectin GSII : pas dans l'appareil de Golgi

XCL1 se retrouve dans les lysosomes

Mécanisme d'endocytose

XCLfacilitates endocytosis.

Endocytose of GFP via XCL : co-localization of XCL and GFP.



XCL

Conclusions :

- L’activité insecticide des champignons supérieurs comestibles provient d’une (ou plusieurs) lectine(s).

- La structure quaternaire de la lectine génère une cavité de 1000 Å3 pouvant contenir des molécules exogènes.

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