

Differences of the nucleolus in 2 species of *Amyclina* (Moll. Gast.)

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Summary. Two *Amyclina* species were examined concerning the more significant anatomo-morphological differences and the different cytological aspects in the nucleoli of growing oocytes.

Recent studies¹ demonstrated that in most Mollusc species the nucleolus of growing oocytes, though it always shows 2 functional phases, manifests characteristics which are peculiar to each species. It was also observed that the nucleolini, i.e. the highly refractive granules which are present in the nucleoli of cells with enhanced protein synthesis, show different morphological aspects within the same systematic group of sea urchins². The purpose of this paper is to describe the morphological differences between the nucleoli of 2 Mollusc species, *Amyclina corniculum* and *Amyclina tinei*; this work is part of studies concerning the body morphology and the oogenesis of these 2 species which are in progress in our laboratory.

Material and methods. The *Amyclina* genus (Gastropoda Prosobranchia) belongs to the Nassariidae family. Only 2 species are present in the Mediterranean sea: *A. corniculum* (Olivi) and *A. tinei* (Maravigna). The former species, which is widely distributed, has been studied by several authors³⁻⁵ under different names. In contrast, the latter, with a very limited distribution, has not been extensively examined and is still considered as a local variety. The specimens examined were collected from the 'montagnole' of lake Faro (Messina), which represents the only habitat for *A. tinei*, and only a temporary one for *A. corniculum*, which usually lives in coastal waters. Fragments of the female gonad fixed in Bouin, Bouin-Hollande with mercury bichloride and 10% formalin were subjected to Mallory's and Dominici's methods. In order to show the presence of nucleolini, fixation in Cajal liquid and subsequent impregnation with 1% platinum chloride⁶ was carried out.

Results and discussion. The most notable anatomo-morphological differences are summarized in the table. As regards the nucleolar apparatus in the 2 *Amyclina* species, some remarkable differences are found. In the *Amyclina corniculum* oocytes it is possible to observe, in the first growing phase, a homogenous nucleolus in which, during the previtellogenesis, a big vacuole already appears. This vacuole gradually becomes larger, so that, in the subsequent vitellogenesis, its main constituents are scarcely present (figure 2).

In oocytes with final yolk globules, the large vacuole appears limited by a continuous or occasionally interrupted line of nucleolar material: on one side of this line, other sausage or semilunar-shaped nucleolar material may remain.

In the younger *Amyclina tinei* oocytes, there is only one homogenous nucleolus; in the successive growing phases, the nucleus, which shows a vesicular aspect, presents 2 nucleoli of different shape and dimensions, initially separated (figure 3) and then tightly joined together. One of these 2 nucleoli is spherical and larger; at the end of previtellogenesis, it already shows a vacuole which gradually enlarges to occupy the nucleolar area almost completely in the successive vitellogenesis.

It can therefore be said that, in both species, in the transitional stage between previtellogenesis and vitellogen-

a **b**

Fig. 1. Ventral view of both *Amyclina corniculum* (a) and *Amyclina tinei* (b) shells.

Fig. 2. Previtellogenic oocyte of *Amyclina corniculum* showing a large vacuole and the part of the nucleolus remaining (arrow). $\times 2400$.

Fig. 3. Nuclear portion of previtellogenic oocyte of *Amyclina tinei* where it is possible to observe 2 nucleoli revealed by platinum chloride impregnation (arrows). $\times 2400$.

Anatomo-morphological differences between *Amyclina corniculum* and *Amyclina tinei*

	<i>A. corniculum</i>	<i>A. tinei</i>
Columellar lip	Small and prominent	Flat and spread out
Shell colouration	Dark brown with clear stripes	Blackish
Height/width ratio	Mean value = 2	Mean value = 1.7
Shape of the operculum	Subovoid with the peristomial side serrated	Trapezoid with the longer sides serrated
Muscular impression on the operculum	Of variable shape	Triangular
Radular formula	1 + 1 + 1	1 + 1 + 1
Size of radular teeth	Lateral teeth, average 83.7 µm Central teeth, average 83.7 µm	Lateral teeth, average 69.3 µm Central teeth, average 83.2 µm
Form of radular teeth	Lateral: 2 large external cusps and one or more small internal cusps Central (rachidian): curved with toothlets varying from 13 to 17	Lateral: bicuspidate Central (rachidian): curved with 9 toothlets

esis, the nucleolar apparatus loses its initial morphological characteristics as a result of vacuolisation processes. The fact that differences in the nucleolus exist between 2 species of the same genus is remarkable. It confirms the results of the studies carried out on nucleoli of other Mollusc species¹ as well as those which demonstrated the different morphological aspects of the nucleolini in 4 species of Echinoidea². However, it must be underlined that in both *Amyclina* species the oocytes show analogous processes as regards the yolk synthesis: in fact, the yolk globules form around the nucleus and then spread throughout the cytoplasm, increasing parallel in size. The final globules, of more or less

similar dimensions in both species (max. diameter 15–16 µm), are sometimes subdivided into 2 portions, an outer and an inner, of which the latter is generally fragmented.

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Etude de l'influence de la tension d'O₂, de CO₂ et de l'anaérobiose sur la morphologie et le nombre des colonies de pneumocoque

Influence of O₂-, CO₂-tension and anaerobiosis upon number and morphology of pneumococcal colonies

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Summary. Cultivation of *Streptococcus pneumoniae* is markedly favored by strict anaerobiosis if compared with relative anaerobiosis.

Les colonies de pneumocoque sont, dans la pratique journalière, presque partout isolées en anaérobiose relative (atmosphère appauvrie en O₂ et enrichie en CO₂). Or Howden², en Angleterre, met en évidence un pourcentage relativement élevé de souches anaérobies strictes. Nous étudions ici le comportement de souches isolées en Suisse par rapport à 3 différents modes d'incubation.

Matériel et méthodes. Les 100 souches de pneumocoque avec lesquelles nous avons travaillé ont été collectionnées dans notre laboratoire; elles ont été prélevées sur 100 patients différents et ont une dizaine d'origines cliniques différentes. Elles ont été isolées le 1er jour de culture sur géloses au sang humain 5% incubées à 37 °C en anaérobiose relative; elles répondent toutes aux critères requis par Lund³.

L'inoculum pour les ensemencements ultérieurs a été préparé dans des sérum-bouillon (SB = trypticas + sérum de veau 15%) et incubé 18 h à 37 °C en anaérobiose relative. Après dilution de ce SB au 1:1000 dans de l'eau distillée nous avons procédé à une numération de germes selon la méthode quantitative habituelle: dilution en tubes et ensemencements sur géloses. Ces géloses ont alors été incubées 24 h à 37 °C, respectivement en aérobie, en anaérobiose relative et en anaérobiose stricte avec adjonction de CO₂ (GasPak® System).

Résultats et discussion. La figure montre clairement l'influence des différents modes d'incubation sur la morpholo-

gie des colonies: l'incubation à 37 °C sans adjonction de CO₂ (aérobie) ne permet qu'à de très petites colonies de se développer. Leur coloration verte caractéristique n'est souvent que peu apparente; ces colonies ne sont pas ombiliquées. L'hémolyse α est mal définie. L'incubation à 37 °C avec adjonction de CO₂ (anaérobiose relative) permet

Comparaison de la croissance numérique du même inoculum de pneumocoque après 24 h d'incubation à 37 °C en aérobie, en anaérobiose relative et en anaérobiose stricte

Croissance en anaérobiose relative*	Nombre de souches	Croissance en anaérobiose stricte*	Nombre de souches
> aérobie	55	> anaérobiose relative	32
= aérobie	32	= anaérobiose relative	48
< aérobie	13	< anaérobiose relative	20**

* > indique que le nombre de colonies est supérieur à la condition indiquée; = indique que le nombre de colonies est égal à la condition indiquée; < indique que le nombre de colonies est inférieur à la condition indiquée.

** dont 12 souches muqueuses sur les 13 que nous avons à disposition.