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Cytochemical localization of surface carbohydrates on mycoplasma membranes[1]

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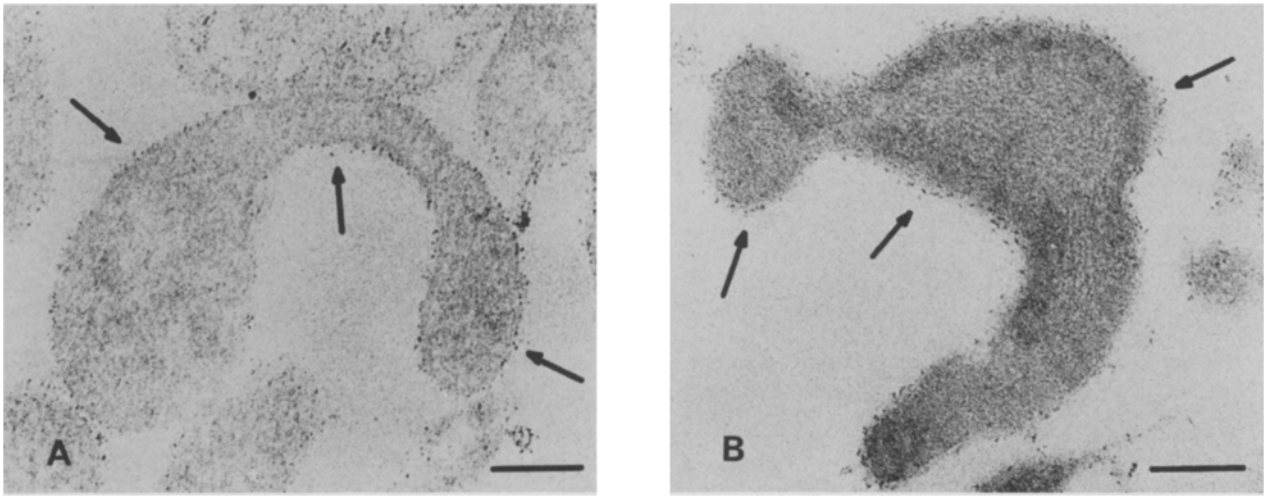
Summary. Surface carbohydrate structures, containing α-D-glucosyl or sterically closely related residues, were visualized on mycoplasma membranes by a cytochemical staining procedure with concanavalin A and iron-dextran complexes.

Mycoplasmas are the smallest microorganisms capable of autonomous growth. They possess no cell wall and intracytoplasmic membranes, but are endowed with a cytoplasmic membrane more stable than that of bacteria[2]. Many morphological and biochemical similarities exist between mycoplasma and mammalian plasma membranes[2]. For instance, in analogy to eukaryotic plasma mebranes, carbohydrates are exposed on the surface membranes of several mycoplasma species[2-5].

Surface carbohydrate structures on mycoplasma membranes were visualized in the electron microscope by applying a recently developed, sensitive staining procedure[6,7], which is based on the finding that concanavalin A (Con A) has at least 2 reactive sites: 1 binds to cellular carbohydrates containing α-D-glucosyl or sterically closely related residues[8], the 2nd acts as a receptor to bind glucose units of exogeneously added, electron-dense iron-dextran complexes.

Materials and methods. Mycoplasma neurolyticum type A (Sabin) Freundt and *Mycoplasma gallisepticum* Edward et Kanarek were kindly provided by J.G. Tully (Bethesda, USA) and E.A. Freundt (Aarhus, Denmark), respectively[3]. The mycoplasmas were grown and harvested, as described previously[5]. Intact mycoplasmas (2 mg of protein) were suspended in 2 ml of phosphate buffered saline (PBS), containing 0.002 M phosphate, pH 7.0, and 0.145 M NaCl, and allowed to react with 1 mg of Con A/ml for 60 min at 22 °C. After centrifugation, the mycoplasmas were washed



Mycoplasma neurolyticum (A) and *Mycoplasma gallisepticum* (B) labelled by the cytochemical staining procedure with Con A and iron-dextran particles. Cells were not contrasted by lead citrate and uranyl acetate. Marker of surface carbohydrate structures uniformly distributed over the entire membrane surface (arrows). Bar, 100 nm.

twice by resuspension in PBS and centrifugation. Coupling to the iron-dextran was performed by suspending the cells in 1 ml of PBS and adding 1 ml of Imferon (Fisons Ltd, Loughborough, England) consisting of 50 mg iron as a complex of ferric hydroxide with dextrans of average mol. wt between 5000 and 7500. The mixture was held for 60 min at 22 °C. The reaction was stopped by dilution, and the mycoplasmas were centrifuged and washed 3 times with PBS. In control experiments, the binding sites of Con A were blocked by the specific inhibitor[8], α-methyl-D-glucopyranoside, final concentration 0.2 M. Thin sections of the cytochemically treated mycoplasmas were examined in the electron microscope[4] without conventional staining by uranyl acetate and lead citrate[6].

Results. After cytochemical treatment, the non-contrasted *Mycoplasma neurolyticum* (figure A) and *Mycoplasma gallisepticum* (figure B) cells showed a dense and homogeneous layer of discrete, electron-dense iron-dextran particles covering the entire membrane surface. Labelling of *M. gallisepticum* was obviously independent of the terminal bleb structure[2]. The cytochemical reaction was completely inhibited by the specific Con A-inhibitor, α-methyl-D-glucopyranoside.

Discussion. High electron-microscopic magnification is necessary for visualization of the electron-dense iron-dextran particles bound to the free valences of Con A which, in a first step, had been bound to mycoplasma surface carbohydrate structures. Due to the small size of the marker molecules, the iron-dextran technique presumably allows a more precise and perhaps stoichiometric demonstration of cellular carbohydrates than can be obtained by labelling with the large Con A-ferritin molecules[5]. On mycoplasma membranes, we regularly observed a lower density of marker molecules than could be detected on mammalian plasma membranes[6,7]. This result is fully consistent with the data of our agglutination experiments with lectins[3].

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Two new polyploid *Xenopus* species from western Uganda[1]

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Summary. 2 new species of the anuran genus *Xenopus* have been found in western Uganda: *X. ruwenzoriensis sp.n.* with the hexaploid chromosome number of 108 in the Semliki Valley, west of the Ruwenzori, and *X. species nova* with the tetraploid chromosome number of 72 in and near lake Bunyoni.

In May 1972 we had the occasion to collect frogs of the genus *Xenopus* (Pipidae, Anura) in the western part of Uganda. 2 of the 4 types encountered displayed the unusually high chromosome numbers of 2n = 75 and 2n = 108, which contrast with the 2n = 36 of most other *Xenopus* species[2]. Both types breed true, giving rise to fertile offspring of both sexes. Moreover, they are distinct on the basis of morphological and biochemical characteristics. Thus, these 2 forms must be considered as taxa in their own right.

Xenopus ruwenzoriensis sp.n. (table, figures 1 and 2). From the Rain forest near Bundibugyo in the Semliki Valley at the foot of the Ruwenzori Mountain (1 °N, 30 °E; altitude 700 m), natives brought us 13 adult *Xenopus* (2♂ and 11♀) of a type similar to *X. fraseri*. 2 of the females differed from the others in their somewhat reddish dorsal colour and some minor morphological characteristics; they possessed 2n = 36 chromosomes and are most likely representatives of *X. fraseri* Boulanger. Attempts to cross these 2 females with males of *X. fraseri* and *X. ruwenzoriensis* were not successful.

The other specimens gave fertile offspring of normal sex ratio, which had, like their parents, a karyotype of 2n = 108 chromosomes. Correspondingly, the DNA content of erythrocytes is 2.6 times that of *X. laevis*[3]. During meiosis, chromosomes generally pair to form bivalents both in oocytes (Müller, unpublished results) and in spermatocytes[2]. These observations show that our small sample belongs to a true-breeding population of hexaploid *Xenopus*; we propose the name *Xenopus ruwenzoriensis sp.n.*.

X. ruwenzoriensis resembles strikingly *X. fraseri*. As in the latter, the prehallux is armed with a claw, tentacle length is at least half the diameter of the smallish eyes. The ventral body surface is whitish grey with few dark spots, while the ventral face of the thigh may be densely spotted on a yellowish background. The back is grey, often with a slightly darker pattern. A dark grey-brown transverse band between or behind the eyes can often be observed; it is most clearly visible in juvenile specimens. As compared to *X. fraseri*, *X. ruwenzoriensis* reaches greater maximal length in both sexes (table). The snout is relatively shorter and the mean number of lateral line organs around the eye is higher.

Xenopus sp. nova. 2 samples of clawed frogs were found around Lake Bunyoni: 3 ♀ and 4 ♂ were trapped by a native fisherman on the eastern shore of Lake Bunyoni near Kyabahinga (altidue 2007 m), the other sample of 28 ♀ and 45 ♂, all juvenile, were taken from an artificial fishpond (altitude 2130 m) near Kashasha (figure 1). The pond contained many hundreds of *Xenopus* tadpoles and newly metamorphosed froglets. Kashasha lies in a valley to the west of Lake Bunyoni, with which it is connected.

The adult *Xenopus sp.n.* (table) is a medium-sized frog without a claw on the prominent prehallux. The dorsal surface is immaculate, of uniformly olive to dull chocolate brown colour. The ventral surface of the belly and thigh is of a striking yellow ochre or dep orange colour, sometimes with small dark pigment spots. However, many specimens are also ventrally unspotted. The eyes are relatively small and far apart, the subocular tentacles are short and covered