The nucleocytoplasmic distribution of 3-0-methylglucose in the amphibian oocyte[1]

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Summary. Ultra-low temperature microdissection was employed to determine the in situ nucleocytoplasmic distribution 3-0-methylglucose in the amphibian oocyte. The nucleocytoplasmic partition ratio, $K_{n/c}$, for this non-metabolizable monosaccharide was 1.54 ± 0.08. The observed asymmetry could be explained by the differential solubility of the solute in the water of the nucleoplasm and the cytoplasm.

Our understanding of nuclear membrane transport properties is hampered by the technological problems associated with the study of these parameters in the in situ preparation. Generally, the study of solute exchange across the nuclear envelope is attempted in small somatic cells in which visualization and monitoring of transport properties is hindered by the resolution of the in situ properties. Such problems have necessitated the use of nuclear isolation techniques which may be subject to internal error resulting from degradation, displacement and rupture of normal structures. Consequently, whenever cells are disturbed, as they are when nuclei are isolated, the question must be asked: Do the membrane transport properties of the isolated nuclei reflect the true in vivo behavior of this organelle.

The development of cryogenic techniques[3–5] has greatly enhanced our abilities to study nuclear function in the living, minimally altered cell, eliminating problems associated with solute redistribution and membrane disruption. In this study, ultra-low temperature microdissection will be employed to characterize the nucleocytoplasmic distribution of the non-metabolized monosaccharide, 3-0-methylglucose (3-0-MG).

Methods. Mature oocytes were isolated from gravid Rana pipiens in frog Ringer's solution containing 24 mM glucose as previously described[6]. The Ringer's solution employed in the incubation experiments contained 24 mM 3-0-MG in substitution for 24 mM glucose. Samples of a [^{3}H]-3-0-MG stock (New England Nuclear, Boston, Mass.) were lyophilized and added to the 3-0-MG Ringer's solution producing a final activity of 50 μCi/ml.

The experimental procedure consisted of: a) incubation of the oocytes in the [^{3}H]-3-0-MG Ringer's solution at 20 °C and b) sampling the oocytes at various intervals of influx by either extraction or microdissection and liquid scintillation spectrometry[4]. The nucleocytoplasmic distribution of solute was determined by employing a microdissection apparatus designed to allow free-hand microdissection of the oocytes on a low-temperature (–60 to –70 °C) dissection stage[4]. In brief, the technique involves isolation of groups of 5–6 oocytes on an aluminium dissection stage, freezing the cells in liquid Freon cooled to –160 °C, and storage in liquid nitrogen. To isolate the nuclei and cytoplasm, the oocyte mountings were inserted in the cold dissection stage and microdissection was done at –60 to –70 °C using clean insulated microtools. The surface of the animal hemisphere was removed by scraping until the nucleus appeared as a small, white hard piece of ice embedded in a dark slightly softer matrix of cytoplasm. The isolated nuclei and cytoplasmic fragments were then isolated in separate foil envelopes, weighed and analyzed as described for whole oocytes.

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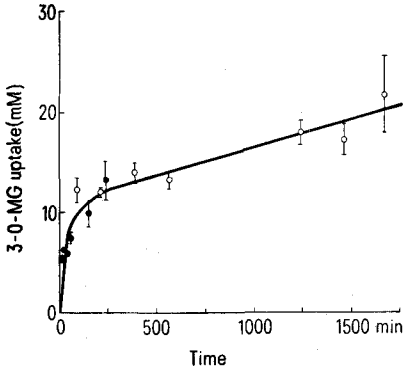

Fig. 1. Effect of time on 3-0-methylglucose (3-0-MG) uptake by 2 populations of amphibian oocytes. Both population A (●; 2.92 ± 0.02 mg wet wt) oocytes and population B (○; 2.10 ± 0.01 mg wet wt) oocytes were incubated at 20 °C in Ringer's solution containing 24 mM 3-0-MG. Each point represents the mean ± SEM of 5 oocytes.

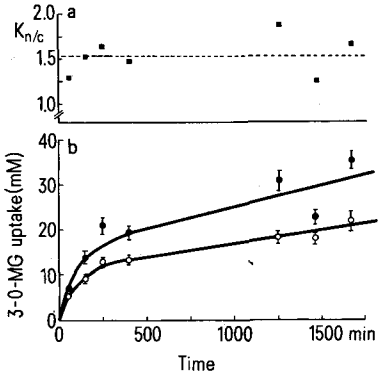

Fig. 2. a) Effect of time on the nucleocytoplasmic 3-0-MG distribution ratio ($K_{n/c}$). Each point represents the mean $K_{n/c}$ determined from 2 oocyte mounting stages each containing 6 oocytes. The mean $K_{n/c}$ over time is 1.54 ± 0.08. b) Effect of time on 3-0-MG uptake into nuclei and cytoplasm isolated from whole oocytes incubated in Ringer's solution containing 24 mM 3-0-MG at 20 °C. Nuclear points (●) represent 2–4 envelopes each containing 4–6 nuclei. Cytoplasmic points (○) represent 5–7 envelopes containing samples of 5–6 oocytes.

Results and discussion. Figure 1 shows the time course of 3–0–methylglucose uptake by 2 populations of mature amphibian oocytes. In both group A (wet weight: 2.92 ± 0.02 mg; % H_2O: 44.8 ± 0.2) and group B (wet weight: 2.10 ± 0.01 mg; % H_2O: 45.7 ± 0.0) solute uptake was gradual, approaching the extracellular concentration of 3–0–MG after approximately 27 h of incubation. In contrast, the uptake of the non-metabolizable amino acid, α-aminoisobutyric acid, exhibits the properties of concentrative uptake and competitive flux inhibition[5]. As illustrated in figure 1, the transport of 3–0–methylglucose across the oocyte plasma membrane appears to be a diffusional process and does not resemble the energy-dependent concentrative uptake of sugars exhibited by the cells of the small intestine and the kidney[7].

The time courses of uptake of this non-metabolizable monosaccharide in the nucleus and cytoplasm as determined by microdissection were similar to that in the whole oocytes (figure 2B). Both compartments exhibited a gradual uptake of 3–0–MG, approaching and exceeding the extracellular concentration after approximately 27 h of incubation. During the 27 h of influx in 24 mM 3–0–MG, the concentration ratios for the nucleus (C_n/C_o) and cytoplasm (C_c/C_o) with respect to the 3–0–MG Ringer's were 1.48 ± 0.08 and 0.92 ± 0.08, respectively.

During the influx the nucleocytoplasmic ratio, $K_{n/c}$, remained constant at 1.54 ± 0.08 (figure 2A). This suggests that any diffusional delay at the nuclear envelope is small relative to that at the plasma membrane and that saturable carriers are not involved in the nucleocytoplasmic transport process. The observed kinetics can be explained by the permeation of 3–0–MG across the nuclear envelope by diffusion processes. The nucleocytoplasmic asymmetry for 3–0–MG is consistent with previous demonstrations that the nuclear envelope is not a permeability barrier for the disaccharide sucrose[8–10]. An exception is the observation that the nuclear envelope of intestinal cells appears to be a barrier to galactose permeation[11].

In the absence of selective mechanisms for sugar permeation and uptake across the nuclear membrane, other processes must exist to explain the observed asymmetries. Horowitz and Moore[12] have proposed that the nucleocytoplasmic asymmetries observed for other solutes, including glycerol[6], sucrose[8,10], α-aminoisobutyric acid[5], inulin[12] and dextran[13] are the result of nonmembrane processes. In particular, the determinants of these nucleocytoplasmic solute asymmetries appear to be equilibrium processes such as macromolecular binding and the related phenomenon of differential solubility in the water of nucleoplasm and the cytoplasm. While this study does not specify the mechanism(s) responsible for the partition of 3–0–methylglucose between the nucleus and cytoplasm, the results can be explained by the partial exclusion of the solute from the water in the cytoplasm.

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Ethanol: Larval discrimination between two Drosophila sibling species[1]

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Summary. Newly hatched larvae of D. melanogaster preferentially migrate to agar containing ethanol, whereas its sibling species D. simulans shows no initial preference. This can be related to the ecological biology of resource utilization in the wild.

Little is known of the ecological biology of the majority of Drosophila species. Even the cosmopolitan siblings melanogaster and simulans, although finding very widespread use in genetical research, have not been studied in great depth ecologically. It has been shown that melanogaster adults and larvae are more tolerant to ethanol in the laboratory and in nature than simulans[2], apparently because the former species is better able to utilize ethanol as a food resource than the latter; melanogaster adults have also been shown to migrate towards wine fermentation tanks during vintage while simulans adults move in the opposite direction[3]. Although resources exploited at the larval stage are of obvious importance for the development of Drosophila species, few reports consider this stage[4]. We report here on a behavioural difference between these 2 species whereby newly hatched first instar melanogaster larvae preferentially migrate to agar containing ethanol, while the movement of simulans larvae is initially independent of ethanol.

10 newly hatched larvae were placed centrally on a Petri dish containing agar; 1 semicircle of the agar contained 6% ethanol. The relative numbers on the 2 sectors were noted for periods up to 2 h. We tested 6 isofemale strains of *simulans* and 5 of *melanogaster* derived from a Melbourne population (10 replicates per strain). There was no overlap across species and means across strains were therefore pooled; they are plotted as mean numbers choosing agar with ethanol up to 120 min from the start of the experiment in the figure.

D. melanogaster larvae showed a clear initial preference for ethanol which slowly diminished with time. The larvae crawled about on the agar presumably in search of food which, apart from the ethanol[5], was not provided; the slow fall in the number on the ethanol-containing agar with time thus appears reasonable. D. simulans larvae on the other hand showed no initial preference, although there was a slow fall in the number of larvae on ethanol-containing agar with time, suggesting that simulans larvae slowly begin to exhibit a tendency towards alcohol avoidance.

These results parallel those acquired for oviposition; melanogaster frequently shows a preference for ethanol-containing media while simulans tends to oviposit on media without ethanol[2]. However, the results are far more un-