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# *Thesis Abstracts*

## Spontaneous mutation related to gene *meth*$G_1$ of *Aspergillus nidulans*

## (Mutações espontâneas relacionadas ao gene *meth*$G_1$ de *A. nidulans*)

*Marta dos Santos Baracho**

Mutations related to gene *meth*$G_1$ of *A. nidulans* were analyzed in order to verify if there is a preferential site where these mutations occur, and to study a mathematical model for the determination of the mutation rate per nucleus per generation, in filamentous fungi. A replica plating technique was used to inoculate, in a single operation, 26 colonies of the strain on Petri dishes containing culture medium. But only the nine central colonies were analyzed for size and volume, number of conidia and nuclei in each colony, viability of conidia, size of the conidial heads and number of conidia in each conidial head. Using this technique, 99 central colonies were analyzed with regard to the appearance of mutation and the number and type of reversions were determined for each colony. The frequencies obtained for each reversion were analyzed in order to verify if they followed a Greenwood and Yule distribution. From the data obtained we may conclude that the mutations analyzed occur preferentially in the sterigma and that, by means of the mathematical model studied, it is possible to determine the mutation rate per nucleus, per generation, in filamentous fungi.

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## The Philadelphia chromosome. Detection by cytogenetics traditional methods and fluorescent *in situ* hybridization (FISH)

## (O cromossomo Filadélfia. Detecção pelos métodos tradicionais de citogenética e hibridação por fluorescência *in situ*)

*Eloisa Auler-Bittencourt**

The Philadelphia chromosome (Ph) resulting from a t(9;22)(q34;q11) translocation is a highly specific finding in bone marrow cells of patients with chronic myeloid leukemia (CML), as it occurs in 90 to 95% of cases. In 5 to 10% of cases, the Ph chromosome is involved in more complex rearrangements, producing the same classic molecular change, but not always being identified by traditional cytogenetics, as it may appear as a normal karyotype or as a different rearrangement. The crucial pathogenetic consequence of this translocation is the formation of a hybrid *BCR/ABL* gene, the product of which is a chimera protein with tyrosine kinase activity. Based on recent evidence, it has been suggested that CML should be defined by the presence of a Ph chromosome (or the *BCR/ABL* rearrangement) and that its absence would be consistent with a different myeloproliferative process. The fluorescent *in situ* hybridization (FISH) method has been used in addition to cytogenetic analysis for the identification of chromosome segments involved in complex rearrangements and has also shown to be a sensitive method for the detection of the *BCR/ABL* fusion, both in metaphases and in interphase nuclei. Treatment with interferon-alpha (IFN-α) induces hematological remission in 60 to 80% of patients with CML in the chronic phase. Patients in which a significant hematological response is obtained show a greater survival rate than those who do not respond to therapy. For cytogenetic analysis, cells in metaphase are necessary, whereas the FISH technique allows detection and quantitative evaluation of the *BCR/ABL* fusion in interphase nuclei. In this study, the analysis of 127 specimens of bone marrow aspirate from 97 patients showed the presence of the Ph chromosome to be the sole chromosome abnormality in 76 patients. Three patients had additional chromosome abnormalities besides the t (9; 22); two patients had complex translocations involving chromosomes 9 and 22, and four had chromosome abnormalities, which did not involve chromosomes 9 and 22. Thirteen patients had normal karyotypes. A total of 40 specimens of bone marrow aspirate from 19 patients were referred for the monitoring of the frequency of Ph chromosome-positive cells after treatment with IFN-α or bone marrow transplantation. The FISH technique using *bcr/abl* probe (Vysis, Inc.) was applied on 12 specimens from nine patients who had shown some extent of a cytogenetic response. The results obtained by the two techniques showed a good correlation, thus suggesting that FISH can be used to monitor the cytogenetic response of patients submitted to treatment with IFN-α.

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## Evolution and population structure in the genus *Gymnotus* (Pisces: Gymnotiformes)

## (Evolução e estrutura de populações no gênero *Gymnotus* (Pisces: Gymnotiformes))

*Flora Maria de Campos Fernandes-Matioli**

Evolutionary and population genetic approaches were carried out in the analysis of species and populations in the genus *Gymnotus* (Gymnotiformes: Gymnotidae). This genus encompasses weakly electric fishes with wide Neotropical distribution. This work was conducted in three main ways: 1) species diversity, geographic distribution and population structure were inferred from the analysis of the nuclear distribution of microsatellites; 2) an interspecific phylogeny involving four *Gymnotus* species was proposed based on the sequences of the mitochondrial genes ND2 and CO1, using parsimony, maximum likelihood methods and statistical tests; 3) coalescent analysis of mitochondrial alleles was performed in two *Gymnotus* species in order to reconstruct their demographic and mutational histories. The patterns of amplified DNA fragments flanked by (GGAC)n microsatellites, obtained by SPAR (single primer amplification reaction), were analyzed from 154 specimens of *Gymnotus* (Pisces: Gymnotiformes) sampled from 20 populations of Southeastern Brazilian basins. The patterns obtained reflect the distribution of simple sequence repeats along the nuclear genome of the specimens. Species-specific patterns of amplification were found, associated with a moderate intraspecific polymorphism. The variation observed enabled the analysis of species diversity and of geographic distribution of *Gymnotus*. In each basin a particular set of polymorphic patterns was found, revealing a population structure that implies restricted gene flow in *Gymnotus*. The hypothesis that the genetic variability involving microsatellite loci may have resulted from concerted evolution associated with molecular drive is proposed. The comparative analysis of 981 nucleotides and their corresponding amino acids, sequenced from the mitochondrial genes ND2 and CO1, in four *Gymnotus* species, using phylogenetic methods, allowed the inference of their evolutionary relationships. In the proposed phylogeny, *G. pantherinus* is located as the most basal species among the taxa studied. *G. carapo* and *G. sylvius* probably are a monophyletic group presenting the most recent divergence. *G. inaequilabiatus* is located as the closest sister group of *G. carapo* and *G. sylvius*. Specimens of Cypriniformes and Characiformes were used as outgroups. The LRT test results indicate that the hypotheses of identical rates of base frequencies can be rejected. Equal rates of substitution, transitions and transversions, and the molecular clock could be rejected as well. The GTR test showed that the topology obtained in the single most parsimonious tree cannot be rejected as null hypothesis. Considering the coalescent process analysis involving *G. carapo* populations from Pantanal do Miranda and Paranapanema basin, it is proposed that these populations cannot be considered as a single panmitic unit. Nowadays these populations are isolated, but sporadic historical migration events between them might have occurred. On the other hand, *G. pantherinus* populations from the basins of Rivers Costeiros and Alto da Serra do Mar seem to have independent demographic histories. In these populations, the most recent common ancestor is placed on a probable ancestral population, which was subdivided. All genealogies presented an exponential distribution of coalescent events, which indicates that no bottleneck or great dispersion events seem to have occurred. The hypothesis of selection was rejected, and the allelic variability observed may be considered to be due to genetic drift, according to the HKA test.

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## Crosses among isofemale lines of *Drosophila eleonorae* (Diptera, Drosophilidae) from three Brazilian caves

## (Cruzamentos entre linhagens de *Drosophila eleonorae* (Diptera, Drosophilidae) de três cavernas brasileiras)

*Anette Hitomi Shoji**

*Drosophila eleonorae* Tosi *et al*., 1990 (Diptera, Drosophilidae) belongs to the *repleta* subgroup of the *repleta* group of the subgenus *Drosophila*. It is a cave-dwelling species which breeds in fresh hematophagous bat (Chiroptera, Phyllostomidae) guano and, as far as it is known, it is restricted to Brazilian caves (from State of Pará to State of São Paulo). It was the purpose of the present work to perform crosses among three isofemale lines from different caves: *Caverna Pedra da Cachoeira* (Altamira, PA), *Gruta do Tobogã* (Cordisburgo, MG) e *Gruta do Fazendão* (Ipeúna, SP) in order to find out any eventual differences regarding these putatively isolated populations. Several markers were analyzed: a) mass mating crosses, b) heterochromatin distribution and morphology of the mitotic metaphase chromosomes, c) banding pattern in polytene chromosomes and d) morphology of aedeagus and spermathecae. The intra- and interstrain crosses were fertile and the offspring of the $F_1$ generation were apparently identical regarding to the distribution of the heterochromatin and to the shape and the basic number of six chromosomes which consist of four telocentrics, one acrocentric and a dot. Moreover, inversions were not detected in the polytene chromosome banding pattern. Concerning the aedeagus, no morphological differences were detected, but among the

spermathecae from distinct localities, significative differences have been observed. The absence of differentiation, except for the latter marker, may be either an indication that some degree of allelic flow does occur among the three apparently allopatrid populations or that the putative geographical isolation is not old enough to have allowed the populations to differentiate, or yet that, on the studied cave environments, the selective pressures might be quite similar. Although just one female specimen of *Drosophila eleonorae* has been collected outside the caves in a banana-baited trap, we believe that there must be some epigean populations allowing some allelic flow among alopatrid populations because neither reproductive isolation nor differentiation regarding four out of five traits was verified. Even though only three caves have been studied, these three isofemale lines are regarded as belonging to the same species (*D. eleonorae*).

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## Karyotypes of species of the *Drosophila tripunctata* group in a secondary semideciduous forest (Diptera, Drosophilidae)

## (Cariótipos de espécies de *Drosophila* do grupo *tripunctata* em uma mata semidecídua secundária (Diptera, Drosophilidae))

*Adriana de Oliveira Pires**

The *Drosophila tripunctata* group, comprising 61 species, is the second largest of the groups of *Drosophila* endemic to the Americas. Although most of the species belonging to this group are reasonably known regarding their morphological features, chromosomally they are greatly unknown. A better knowledge of these species concerning their karyotypes will likely make the *tripunctata* group a suitable material for studies aiming at understanding the speciation processes occurring in the tropics. The mitotic metaphase plates of larval neuroblasts were analyzed for eleven species of *Drosophila* (including two undescribed ones) collected at the *Reserva Florestal da Cidade Universitária "Armando de Salles Oliveira"*, São Paulo City, State of São Paulo, Brazil. Ten out of the eleven species have 2n = 12 chromosomes and just one (*D. unipunctata*), 2n = 10. The analyzed species and their male karyotypes are as follows. *D. bandeirantorum* = 4 rod-shaped chromosome pairs, a pair of dots, one V-shaped X and one J-shaped Y; heterochromatic are the pericentric regions of the rod-shaped pairs, the Y, one arm of the X and the pericentric region of the other arm. *D. bifilum* = 4 rod-shaped pairs, a pair of dots and one V-shaped pair of sexual chromosomes; heterochromatic are the Y, one arm of the X and the pericentric region of the other arm. *D. mediopicta* = 4 rod-shaped pairs, a pair of dots and one V-shaped pair of sexual chromosomes; heterochromatic are the pericentric regions of one of the rod-shaped pairs, the Y, one arm of the X and the pericentric region of the other arm. *D. mediopunctata* = 4 rod-shaped pairs, a pair of dots, one rod-shaped X and one V-shaped Y; heterochromatic are the Y and the pericentric region of the X. *D. mediosignata* = 5 rod-shaped pairs and a pair of dots; heterochromatic are the Y and the pericentric region of the X. *D. platitarsus* = 4 rod-shaped pairs, a pair of dots, one V-shaped X and one J-shaped Y; heterochromatic are the pericentric regions of the rod-shaped pairs, the dots, the Y, one arm (interstitial heterochromatin) of the X and the pericentric region of the other arm. Although *D. roehrae, Drosophila* sp. U3 and *Drosophila* sp. V3 are distinguishable from each other, their metaphase plate consists of 5 rod-shaped pairs and a pair of dots; heterochromatic are the Y, the pericentric regions of the rod-shaped pairs, including the X. *D. trifilum* = 5 rod-shaped pairs and a pair of dots; heterochromatic are the dot pair, the Y, the pericentric region of the X. *D. unipunctata* = one pair of V-shaped, 2 rod-shaped pairs, a pair of dots and one sexual J-shaped pair; heterochromatic are the Y and the short arm of the X. The karyotypes of four species (*D. bifilum*, *D. mediopicta*, *D. platitarsus* and *D. trifilum*) are described for the first time. The karyotypes of the remaining seven species are redescribed and compared to those already reported in the literature.

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## Prader-Willi and Angelman syndromes: chromosome analysis, the spectrum of the clinical variability and the study of marker chromosomes

## (Síndromes de Prader-Willi e Angelman: análise cromossômica, espectro da variabilidade fenotípica e o papel dos cromossomos marcadores)

*Monica Castro Varela**

The Prader-Willi (PWS) and the Angelman (AS) syndromes are clinically distinct developmental and neurobehavioral disorders that result from the loss of imprinted gene expression within chromosome 15q11-q13. PWS patients exhibit neonatal hypotonia with poor suck and failure to thrive; hyperphagia, beginning typically at 1-6 years of age; severe obesity; mild mental retardation; hypogonadism and characteristic facies and behavior. AS patients have hypotonia, psychomotor development delay, severe mental retardation, absence of speech, typical happy

disposition with outbursts of laughter, ataxia, seizures, microcephaly, macrostomia and prognathism. In this work we studied 18 patients with PWS, 20 with AS, 3 with supernumerary marker chromosomes (SMC), and their parents, by GTG banding and FISH techniques: 14 PWS patients showed deletion and 4 disclosed normal results by FISH with SNRPN probe; 16 cases with AS disclosed deletion while 3 showed normal results, and 1 had a translocation [t(15;15)] as detected by FISH with GABRB3 probe. G-banding analysis was less sensitive for deletion detection but useful in demonstrating other cytogenetic alterations. FISH technique was able to detect deletion in about 78% of the PWS and AS patients. FISH using the α-satellite probe (D15Z) and single-copy sequences (GABRB3 and SNRPN) identified the SMC as inv dup(15). Clinical features of 63 patients (35 PWS, 25 AS and 3 inv dup(15)) were described and comparisons with literature data were performed. The 3 inv dup(15) patients had delayed motor development, mental retardation, ataxic gait, seizures and some typical facial features. Comparing the clinical data of 8 UPD with the 27 deletion PWS patients we observed that the age of diagnosis and the birth weight were higher among deletion patients. In addition, our data confirm an increased maternal age in the UPD group. Comparing the clinical findings from 19 UPD AS patients (15 previously published and 4 of our sample) with those 21 deletion AS patients we observed that in the UPD group the age of diagnosis was higher, microcephaly was less frequent, children started walking earlier, epilepsy started later and weight above p75 was frequently reported. Complete absence of speech was more common in the deleted group than in the UPD patients, where half of the children were able to say a few words. Cytogenetic studies by FISH with commercial probes proved to be efficient for the diagnosis of the PWS and AS patients with deletion, but to disclose other genetic mechanisms and for appropriate genetic counseling DNA research is demanded.

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## Popcorn (*Zea mays* L.) genotypes'adaptability and stability evaluation in Brazil center and south regions

## (Avaliação da adaptabilidade e estabilidade dos genótipos do milho de pipoca (*Zea mays* L.) nas regiões centro e sul do Brasil)

*Eliana C. Gruszka Vendrusculo**

Adaptabilty and stability of 15 popcorn genotypes (*Zea mays* L.) were evaluated in 19 areas of central and southern regions of Brazil, in the year of 1990/91. Yield data were used to compare the methodologies that measure phenotypic stability, as following: Traditional (Yates and Cochran (1938)), Plaisted and Peterson (1959), Wricke (1965), Finlay and Wilkinson (1963), Eberhart and Russell (1966), Tai (1971), Verma *et al*. (1978), Cruz *et al*. (1989) and Huehn (1990a). The results obtained in this study showed that the Traditional methodology identified low productive, but stable genotypes, showing the association between low production and stability. The Plaisted and Peterson (1959) and Wricke (1965) methodologies reported the same results for the most productive and stable genotypes. Among all the methods, the ones where the analysis of linear regression used was the Eberhart and Russell (1966) methodology would be the most indicated, due to high facilities of application and the bigger number of information offered. The methods of bi-partitioned regression analysis were equally efficient in selecting genotypes in favorable environments. Huehn (1990a) methodology identified low productive genotypes as the most stable. As a result, high production genotypes were identified, but there were not high popping expansion genotypes. The genotypes UNB-2, CMS-43, CMS-42 and Colorado Pop1 had the highest mean yields in favorable environments, indicating an answer to the improvements in these environments. The genotype Composto Indígena can be considered as having an average adaptability, but not stable performance. Among all genotypes, Colorado Pop1 stands out as the unique genotype which high yield and good popping expansion were associated. In addition, it is recommended to favorable environments and it has a good stable performance.

**1997. Pós-Graduação em Melhoramento Genético Vegetal, Universidade Estadual de Maringá, Maringá, PR, Brasil. Master's thesis. Orienting Professor: Dr. Carlos Alberto Scapim.*

## Study of the variations of the FANCA gene of Fanconi anemia

## (Estudo das variações do gene FANCA da anemia de Fanconi)

*Neiva Isabel Rodrigues Magdalena**

Fanconi anemia (FA) is an autosomal recessive disease, characterized by blood and bone marrow abnormalities starting at a median age of 7 years, associated with various congenital malformations and/or anomalies detected in the patient's physical examination, and with cytogenetic studies showing spontaneous or induced chromosomal breaks. There are eight complementation groups already identified, confirming the existence of genetic heterogeneity. Three of the eight genes responsible for this disease were recently cloned (FANCC, FANCA and FANCG genes). Gene FANCC is responsible for about 10% of the cases and gene FANCA for about 65% of the cases of this disease. In the present work, we studied a group of

30 Brazilian individuals diagnosed with FA and control individuals from the normal population, using the genomic amplification of the 43 exons (and part of the introns) of gene FANCA by PCR and subsequent SSCP analysis and automated DNA sequencing. Thirty-seven variants in gene FANCA were identified, including 13 idiomorphic mutations and 24 polymorphic mutations. Eighteen of the 30 individuals (60%) had idiomorphic mutations in gene FANCA, and the idiomorphic mutation 3788-3790del was found in 9 of the 30 individuals (30%). Seventeen patients with known FANCA idiomorphic mutations, their parents, and 52 individuals from the normal population were studied using ARMS assay to study eight of the polymorphic mutations of the FANCA gene and to identify their haplotypes. Haplotype A,G,T,T,G,G,C,T was the most frequent haplotype found in all the groups studied. This haplotype was found in 68% of the patients, in 50% of the patients' parents and in 36% of the individuals from the normal population. All the patients found to have the 3788-3790del idiomorphic mutation in gene FANCA that could have their haplotypes determined were found to have only one type of haplotype, which was haplotype A,G,T,T,G,G,C,T.

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## Applicability of molecular biology techniques to populational screening of FRAXA and FRAXE mental retardation

## (Aplicabilidade de técnicas de biologia molecular na triagem populacional do retardado mental FRAXA e FRAXE)

*Milene Vianna Mulatinho**

Individuals with mental retardation (MR) are a heterogeneous group. Recent advances in molecular genetics techniques have enabled us to understand more about the molecular basis of several genetic syndromes associated with MR. Of the 120 known XLMR disorders, 53 have been mapped, and 22 genes have been cloned, two of them were *FMR1* and *FMR2* genes, associated with fragile X syndrome and FRAXE mental retardation, respectively. The aim of this work was to confront the Southern blot and polymerase chain reaction (PCR) techniques, comparing their efficiencies on population screening, and, simultaneously, to investigate the GCC amplifications at the FRAXE locus. In this study, we surveyed 85 institutionalized individuals with severe mental retardation, 38 males and 47 females, by both molecular techniques to detect amplifications in CGG (*FMR1*) and GCC (*FMR2*). Through this work we implanted in our laboratory two molecular protocols appropriated to population screening of FRAXA and FRAXE mental retardation. We validated these two methodologies by the application in the sample studied. We had 100% of agreement in the results obtained by PCR and Southern blot techniques in all the 38 males evaluated, considering the PCR an adequate protocol for screening of males with mental retardation. The use of Southern blot is still necessary for the decisive diagnosis of the fragile X syndrome. No FRAXA and FRAXE mutations were found in the *FMR1* and *FMR2* genes, reinforcing the prevalence of fragile X syndrome among non-institutionalized individuals with mild to moderate MR. To exclude chromosomal abnormalities associated with mental retardation as a possible cause of the phenotype in these individuals, the G-banded chromosome analysis was carried out in all patients. In 8% of the cases a chromosomal etiology could be demonstrated.

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*1999. Departamento de Biologia Celular e Genética, Instituto de Biologia Roberto Alcântara Gomes, Centro Biomédico, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, RJ, Brasil. Marter's thesis. Orienting Professors: Dra. Márcia Mattos Gonçalves Pimentel (IBRAG/UERJ) and Juan Clinton Llerena Jr. (IFF/FIOCRUZ, RJ).

## Molecules involved in the cellular interactions of early amphibian development

## (Interações celulares no desenvolvimento dos embriões de anfíbios: moléculas envolvidas)

*Manuel Javier Aybar**

In the early amphibian development, gastrulation is accomplished by active cell movements such as the migration of mesodermal cells, the epibolic movement of the ectodermal wall and the convergence-extension of the marginal zone. In all these events, cell morphology, attachment, migration and differentiation may require adhesion as well as cell-cell and cell-environment interactions. Although there is considerable information indicating that cell surface and extracellular glycoproteins play significant roles in these processes, other cell surface components such as glycosphingolipids (GSL) have not been widely studied as yet in cell-cell and cell-extracellular matrix (ECM) interactions during embryogenesis.

The presence and participation of GSL during early amphibian development of *Bufo arenarum* embryos were investigated. We established the qualitative and quantitative changes in the ganglioside composition of the blastula and gastrula embryos. The analysis of ganglioside abundance indicates that the "a" and "b" synthesis pathways perform similar biosynthetic activities in the blastula stage, in contrast to the gastrula stage in which a marked predominance of the "a" pathway occurred. Using radioactive precursors we established that ganglioside synthesis be-

gan at the blastula stage and reached a maximum during gastrulation (stages 10-12) while neutral GSL synthesis presented a slight gradual increase, the former being quantitatively more significant than the latter. Using different specific inhibitors of GSL synthesis (PPMP and fumonisin B1) we determined that when biosynthesis is significantly reduced, gastrulation is blocked. The scanning electron microscopy morphological analysis of arrested embryos evidenced the inhibition of morphogenetic movements since no radial interdigitation, epiboly of the ectodermal wall, mesodermal cell migration or convergence-extension of marginal zones could be observed. The analysis of mesodermal cell morphology in those embryos showed a severe decrease in the number and complexity of cellular extensions like filopodia and lamellipodia. These cells also lost their cell-cell and cell-ECM contacts. This observation was confirmed by studying *in vitro* cellular adhesiveness to the native ECM substrate.

The spatio-temporal expression of GM1 ganglioside and polygangliotetraosyl ceramides (pGTC) was investigated by wholemount immunocytochemistry using cholera toxin B subunit (CTB) and an affinity purified human anti-GM1 antibody. The pGTC were detected as GM1 after treatment with neuraminidase. Blastomeres from the inner surface of the blastocoelic roof (BCR) of blastula embryos were GM1 and pGTC positive. At mid-gastrula stage, embryos showed an increased labeling on the inner surface of BCR.

In order to establish whether the GM1 ganglioside was involved in the gastrulation processes, CTB, anti-GM1 antibodies and anti-GM1 Fab' fragments were microinjected into the blastocoel cavity of blastula embryos. Treatment with the probes blocked gastrulation. Scanning electron microscopy analysis of blocked embryos revealed that mesodermal cell migration, radial interdigitation and convergent extension movements were affected. The blocking of gastrulation was correlated with the absence of fibronectin and EP3/EP4 on the inner surface of blastocoelic roof of CTB- or anti-GM1-treated embryos. Results show that the GM1 ganglioside is differentially expressed by embryonic cells and participates in the morphogenetic processes of amphibian gastrulation.

The participation of GM1 ganglioside in mesodermal cell adhesion was evaluated. Using both probes we established that this ganglioside is relocated during the adhesion of mesodermal cells. GM1 is distributed preferentially in the cellular extensions such as filopodia and lamellipodia during the cell adhesion process. CTB and anti-GM1 probes inhibited the adhesion and spreading of early gastrula mesodermal cells on native ECM, EP3/EP4, FN and collagen substrates suggesting the involvement of GM1 in these processes.

Our results suggest that glycosphingolipids and particularly gangliosides participate in *Bufo arenarum* gastrulation, probably through their involvement in cell adhesion events. However, further studies will be necessary to provide deeper insights into the involvement of gangliosides in the complex molecular mechanisms of amphibian gastrulation.

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