teiligt ist. Wie die Figuren 1 und 2 zeigen, besteht kein Zweifel an der Identität des Chromosoms Nr. 1. Die Bänderungsstrukturen der kleinsten Chromosomen lassen aber den Schluss zu, dass das 25. Chromosom an der Translokation der untersuchten Halbgeschwister beteiligt war (Figuren 2 und 3). Beide Bullen stammten von einer Kuh, der Vater des in den Figuren 1 und 2 gezeigten Translokationsbullen besitzt den normalen Karyotyp (Figur 4), die Mutter der beiden Bullen wurde kurz nach der Geburt des zweiten Bullen geschlachtet und konnte nicht mehr untersucht werden. Es ist aber zweifelsfrei, dass die Mutter die Trägerin des Translokationschromosomes war und an beide Söhne das abnorme Chromosom weitergegeben hatte. Eine zusätzlich untersuchte Tochter dieser Kuh zeigte den normalen Karyotyp, so dass es sich bei der Mutter um eine heterozygote Form handelte.

Bei der zweiten Rasse wurde die Translokation an Kreuzungstieren der Versuchstierherde des Lehr- und Versuchsgutes Wildschwaige gefunden, wobei ein durch künstliche Besamung eingesetztes Vatertier der Braunviehrasse zwei Nachkommen mit derselben Translokation zeugte. Die Mütter dieser Tiere (Schwarzbunt und Pinzgauer) zeigen den normalen Karyotyp. Zusätzlich untersuchte und in der Zuchtwertschätzperiode befindliche Reinzuchtsöhne desselben Bullen der Braunviehrasse zeigten ebenfalls das Translokationschromosom (Figuren 5 und 6). Der Vererber dieser Chromosomenanomalie war zum Zeitpunkt dieser Erkenntnis bereits geschlachtet, hatte jedoch zahlreiche Nachkommen hinterlassen. Die Untersuchung des Vaters dieses Vererbers ergab einen normalen Karyotyp, so dass entweder die Mutter das Translokationschromosom besessen haben musste oder diese Chromosomenanomalie bei dem betreffenden Bullen neu aufgetreten war.

Die beschriebene 1/25-Translokation hatte bei dem erwähnten Muttertier keinen erkennbaren negativen Einfluss auf die Fruchtbarkeit, da die Kuh jeweils bei der ersten Besamung sechsmal konzipierte. Die zweite Translokation, sie ist zum Typ der 1/29-Translokation zu zählen, soll nun auf Grund der vielen verfügbaren Nachkommen in möglichst vielen Kriterien untersucht werden.

Die unterschiedliche Einschätzung verschiedener Autoren über die Auswirkung von Translokationen auf die Fruchtbarkeit und einiger Leistungskriterien könnte durch die Beteiligung verschiedener Chromosomen am Translokationsgeschehen erklärt werden.

## The Reduction of Genic Activity in the Tetraploid Amphibian *Odontophrynus americanus* is not due to Loss of Ribosomal DNA

J. Schmidtke, W. Beçak[1] and W. Engel

*Institut für Humangenetik und Anthropologie der Universität, Albertstrasse 11, D-78 Freiburg im Breisgau (German Federal Republic, BRD), 20 August 1975.*

*Summary.* Genic activity in tetraploid members of the amphibian species *Odontophrynus americanus* is reduced to that of diploid ones. Loss of ribosomal genes, a mechanism suggested by others as a means of decreasing genetic activity, could be ruled out. The diploids and the tetraploids have almost identical proportions of their genomes complementary to (28s + 18s) ribosomal RNA.

Within the anuran species *Odontophrynus americanus* (Ceratophrydidae) diploid and tetraploid populations exist[2–4]. Diploid animals have a nuclear DNA content of 3.6 pg, while the tetraploids have 7.1 (own unpublished data). Comparative measurements of erythrocyte volume and hemoglobin content per cell, lactate dehydrogenase activity in heart muscle tissue[5], and RNA-content per kidney cell[6], yielded essentially identical values in the tetraploids and the diploids of either *O. americanus* or the close relative *O. cultripes*. Obviously, gene action in these species is not in proportion to nuclear DNA content. It is assumed that in the tetraploids a control system is established which reduces genic activity to the level of the diploids. The acquisition of such a regulatory mechanism would enable the tetraploid organism to escape from the disadvantages of elevated rates of synthetic activity (e.g. large cell size[7,8]), while the advantages of excess DNA are sustained (e.g. higher polymorphism rate, and, in consequence, a wider ecological range[9,10]).

Various observations in animals[11] and plants[12,13] suggest selective loss of (28s + 18s) ribosomal RNA genes as a possible means of decreasing genetic activity. In order to test whether such a mechanism might explain the 'diploidization' of genic activity in the tetraploid *Odontophrynus*, we have determined the relative proportions of DNA complementary to (28s + 18s) ribosomal RNA in the diploid and the tetraploid *O. americanus* by molecular RNA-DNA-hybridization experiments.

*Materials and methods.* Blood aliquots of 4 adult specimens each of the diploid and the tetraploid *O. americanus* were pooled in 0.65% NaCl-0.1% heparin. Cells and nuclei were lysed with Triton-X-100 (final concentration about 0.1%). Isopropanol was slowly added to the gelatineous mass. The resulting precipitate was transferred to 75% ethanol – 0.1 *M* NaCl for several min

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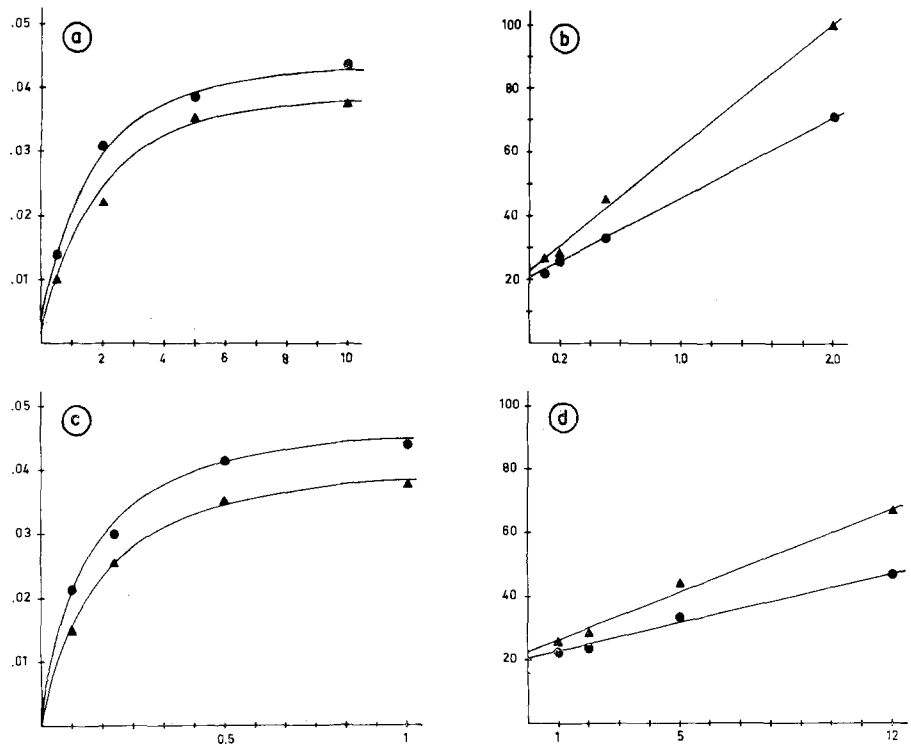


Saturation curves and reciprocal plots of hybrid formation of Hela rRNA with DNA of *O. americanus* 2 *n* (▲) and *O. americanus* 4 *n* (●). Each symbol represents the mean of duplicate determinations. a) Abscissa: concentration in μg/ml incubation mixture of $^3$H-(28s + 18s)rRNA; ordinate: % hybridization (μg RNA bound to 100 μg DNA); incubation time was 1 h. b) Reciprocal plot of (a). c) Abscissa: time (h); ordinate: % hybridization; RNA concentration was 10 μg/ml. d) Reciprocal plot of (c). With respect to RNA input, the saturation plateau was attained at 5 μg/ml (Figure a) and, with respect to time, already after 1/2 h (Figure c). The reciprocal plots yield identical values in either type of saturation experiment (Figures 1 b and d).

and subsequently air-dried on filter paper. From the air-dried precipitate, DNA is extractable even after week-long storage. The precipitate was suspended in water and treated overnight at room temperature with pronase (Merck, 70,000 PUK/g), $CaCl_2$, and LiCl (final concentrations 0.25 mg/ml, 5 m*M*, and 1 *M* respectively). Sodium dodecyl sulfate (final concentration 0.5%) was added, and the mixture was allowed to incubate several hours at 37 °C, followed by excessive treatments with phenol in the presence of m-cresol and 8-hydroxyquinoline[14] and with chloroforme[15]. The DNA was denatured with KOH and finally loaded on Sartorius nitro-cellulose membrane filters (20 μg/filter). DNA-RNA hybridization was performed in 6 SSC – 50% formamide at 65 °C essentially as described by Birnstiel et al.[16] with $^3$H-labelled (28s + 18s) Hela rRNA (specific radioactivity 40,000 cpm/μg), which was a generous gift of Dr. W. Krone, Ulm[15]. In the light of previous demonstrations of effective specific binding of human DNA and amphibian rRNA[17], the use of non-homologous RNA for our hybridization experiments appeared to be justified.

*Results and comments.* The results summarized in the Figure show that the relative proportion of the genome complementary to (28s + 18s) ribosomal RNA is essentially the same in the diploid and in the tetraploid sample. Extrapolation of the reciprocal plots at infinite RNA-concentration and incubation time yield saturation values of 0.043 and 0.049 μg RNA bound to 100 μg DNA, respectively. Thus, the tetraploid is endowed with about twice the number of rRNA genes of the diploid. Therefore, the phenomenon of diploidization of genic activity in the tetraploid cannot be attributed to selective loss of ribosomal DNA.

These results correspond with findings in diploid and ancestrally tetraploid species of the fish family Cyprinidae. While genetic activity is similarly diploidized in the tetraploids[18], the number of (28s + 18s) rRNA genes is in proportion with ploidy[19]. However, diploid and tetraploid carp-like fish have nucleoli (measured in epithelial cells) of very similar size[19]. This suggested to us that the diploidization of the tetraploid was perhaps effected by diminishing transcriptional activity of the ribosomal genes. A similar mechanism, as already suggested, might likewise be responsible for the reduction of genic activity in the tetraploid *O. americanus*[5,6]. This will be studied subsequently[20].

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