

Letter to the Editor

LACK OF EXPERIMENTAL SUPPORT FOR KUZNETSOV'S CRITICISM OF BIOLOGICAL EVOLUTION

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Sir,

Some time ago I became aware of an article in this journal by Kuznetsov entitled: "*In vitro* studies of interactions between frequent and unique mRNAs and cytoplasmic factors from brain tissue of several species of wild timber voles of Northern Eurasia, *Clethrionomys glareolus*, *Clethrionomys frater* and *Clethrionomys gapperi*: A new criticism to a modern molecular-genetic concept of biological evolution" (1989, **49**, 43–59). The article raised my concern because of its unusual approach, its superficially demonstrated results, its surprising conclusions, and the many references cited in unfamiliar journals.

As the article was rather unclearly written, I summarize here the main points. Briefly, messenger ribonucleic acid (mRNA) was isolated from three species of wild timber voles considered to belong to the same genus and, therefore, to be evolutionarily closely related. It was found that each of these species has a small ribonucleotide that inhibits translation of mRNA from the two other species (but not mRNA from the same species from which the ribonucleotide was purified). In contrast, the ribonucleotide did not inhibit translation of more distantly related rabbit globin mRNA or human histone mRNA. Kuznetsov interpreted this as "a new criticism to a modern molecular-genetic concept of biological evolution" (see title) and expressed his belief that the inhibitory factor serves to maintain constancy of species (p. 55). This may be used as an argument supporting "the general creationist concept on the problems of the origin of boundless multitudes of different and harmonically functioning forms of life" (p. 45). In fact, Kuznetsov explicitly claimed that his experiments addressed the phenomenon of evolution: "... to assess the possibility for the translation control to be as an evolutionary or antievolutionary factor" (p. 45). Indeed, it was a "creationist" who brought this article to my attention, using it to argue that there is scientific evidence against evolution (macro-evolution). I summarize here my critique of this article and conclude that Kuznetsov's methodology is inappropriate, that the method references—as well as other references—are questionable or even incorrect, that Kuznetsov's results are insufficiently documented, and that his conclusions are therefore unfounded and cannot be used as evidence against evolution.

The Method and Results sections contain not a single figure that documents the quality of the mRNA used nor of the protein products claimed to result upon translation of this

Correspondence to: Dan Larhammar, Department of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden

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mRNA. The mRNA from *Clethrionomys glareolus* (mRNA₁) was conjugated to BSA according to F. L. Solvarssen and B. Hjerten (1974). This reference was listed as to be published in *Uppsala University Research Reports*, 123, 88–96. This is the university where I have been working since 1980. No such journal could be identified at this university. Moreover, no persons with these names are currently known here. The unusual name of Hjertén is carried by Prof. Stellan Hjertén (Dept. of Biochemistry) who is unfamiliar with both the cited article and its author, B. Hjerten. Finally, neither Solvarssen nor B. Hjerten could be found in the Uppsala University directory (checked time period 1971–1976).

The mRNA₁/BSA conjugates were used to immunize rabbits by administration according to Lokmediani et al. The reference list contains a single-authored paper by R. O. Lokmediani in *Acta Allergologica*, 4, 68–85, 1973. This journal is neither included in Medline nor in CASSI (Chemical Abstracts Service Source Index). A journal called *Allergologica Acta* exists but its volume-year assignments do not match those ascribed to *Acta Allergologica* by Kuznetsov.

Kuznetsov reported purification from rabbit blood serum of IgM and IgG antibodies directed to mRNA by a procedure of Shatsky and Bogdanov described in *Immunochemical and Immunocytological Methods*, 11, 333–349 (1984). This journal too has not been found. The purified antibodies were claimed to be “mRNA₁-selective,” but no experimental evidence for this was presented. Subsequently, Fab fragments were isolated from these antibodies according to Beaud and Chantrennes, (*International Journal of Applied Immunology and Immunochemistry*, 19, 1–12, 1987). This journal, too, could not be found in Medline and CASSI. Finally, the Fabs were immobilized on CNBr-Sepharose particles as described by Kuznetsov and Traichev (*Biotechnologica Acta*, 7, 45–62, 1985). This journal too could not be found nor was the cited paper by Kuznetsov and Traichev included in Kuznetsov’s list of publications for 1982–1989 that was made available to me by a Swedish “creationist”.

Kuznetsov wrote “Thus, owing to affinity chromatography on FaB-mRNA₁-Sepharose columns, it should be possible and not difficult to separate unique, i.e., species-specific, mRNA sequences from the sequences which frequently exist in mRNAs of different organisms, so called repeated mRNA chains (Kuznetsov & Dunlop, 1986)” (p 47). Because I failed to comprehend this sentence, I wished to turn to the cited reference in *Comparative Biochemistry, Biophysics and Genetics*, 33, 669–678 (1986). However, I have also not managed to find this journal. This article, too, is missing from Kuznetsov’s list of publications provided by the aforementioned “creationist”.

Columns with anti-mRNA₁ Fabs were applied with mRNA for either of the three species of voles or either of three control RNAs; rabbit globin 9S mRNA, human histone 11S mRNA, and *E. coli* 5S rRNA. RNA was eluted by an “equilibrium medium” at 25°C (to give mRNA₁-el, mRNA₂-el, etc.) and purified according to Korn, Hummerstihl and Blotter, *Methods and Approaches in Clinical Chemistry and Immunochemistry*, 34, 479–688 (1985). This is another unidentified journal or book. The mRNA was not investigated qualitatively, only quantitatively (Table 2) by translation in a standard (commercial) cell-free translation system from rabbit reticulocytes.

As expected, all mRNA from species 1 was found to bind to the column. For species 2 and 3 approximately half of the mRNA could be eluted at 25°C. When mRNA₂-el was translated in the presence of its own PMS (PMS₂), the ³⁵S-methionine-containing protein gave 123,887 cpm (counts per minute; see Table 1B). However, when mRNA₃-el was translated in the presence of PMS₂, only 3,076 cpm were recorded. Kuznetsov concluded that PMS₂

inhibits translation of mRNA from species 3. Similarly, translation performed in the presence of PMS₃ gave high incorporation of ³⁵S-methionine for its own mRNA but not for that from species 2. PMS₁ seemed to inhibit translation of both mRNA₂-el and mRNA₃-el. In contrast, neither of the three PMS preparations inhibited the translation of affinity-purified mRNA for rabbit globin and human histone. Further, neither of the three PMS extracts inhibited translation of the flow-through from the affinity chromatography, i.e., the unbound mRNA. Remarkably, the standard error from seven separate experiments was often in the narrow range of 0.5–1%.

Thus, the cytosol preparations (PMS) would seem to inhibit translation of "species-specific" mRNA from the two closely related vole species but to allow translation of two distantly related (rabbit and human) mRNAs. Kuznetsov then proceeded to explore the nature of this inhibitor and concluded that it is a small RNA molecule (< 1.0 kD). This led Kuznetsov to suggest that such inhibitory molecules exist in order to preserve the constancy of species.

Since the experimental approach used by Kuznetsov is extraordinary and obscure (and the pertinent references could not be found), these are a few of my major scientific objections and reservations: 1) The concept of injecting mRNA molecules into rabbits in order to obtain antibodies disregards the extreme sensitivity of mRNA to degradation by ubiquitous ribonucleases. 2) The antiserum was raised to polyadenylated mRNA from species 1, yet it failed to bind some polyadenylated mRNAs from the other species. 3) There is no way to discriminate distinctly between "species-specific" and "repeated" (shared between species) mRNAs. Sequence variability between species will form a more or less continuous spectrum with some almost completely identical sequences, some rather variable, and some intermediate. 4) The complex vole mRNA populations should not be directly compared with the single mRNAs from the more distantly related species, i.e., rabbit globin mRNA and human histone mRNA. 5) No qualitative analysis of the translation products was done.

Finally, one of the cited references is an article ascribed to H. V. Hydén (1988) and said to be published in *Scandinavian Archives of Molecular Pathology*, entitled Messenger RNA in the learning of the animals and in the different types of responses to a stress and mutagenic effects in vivo. After unsuccessfully having sought this reference, I contacted Prof. Holger V. Hydén (Gothenburg, Sweden) who is a member of the Editorial Board of *The International Journal of Neuroscience*. Prof. Hydén states that he has written no such article and that he, too, is unaware of the journal *Scandinavian Archives of Molecular Pathology*.

To summarize, Kuznetsov's experimental concept is obscure, his approach goes against established scientific experience and his claimed results are not qualitatively demonstrated. The key methodological references cited by Kuznetsov have not been published in journals listed in Medline or CASSI. These, as well as many other, references are afflicted with complications: some authors could not be found, one author has not written the article ascribed to him, many articles have obvious grammatical errors in their titles, etc. I conclude that Kuznetsov's critique of "a modern molecular-genetic concept of biological evolution" has no scientific basis whatsoever.

Dan Larhammar, Ph.D.
associate professor