Serbian molecular biologists in Regensburg

Miodrag Guzvic
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Molecular Biologist

What my friends think I do
What my mom thinks I do
What society thinks I do

What my boss thinks I do
What I think I do
What I actually do
Why "molecular biologists"

• Molecular biology is increasingly becoming the part of almost all domains of classical biology
  • Still, most of research is related to human health
• Modern, dynamic, exciting, rewarding,…
• One of the key problems: it is very expensive field of science
conversion of 1 μg of DP-NH 2 to DPN per min. by 10^10 erythrocytes at 37°C under our assay conditions. The results are summarized in Table 1. All the red blood cell endonucleases from patients with hereditary spherocytosis were either normal or elevarated. All the other glycolytic enzymes, as well as glucose-6-phosphate and 6-phosphogluconate dehydrogenase, were examined either singly or in multiple steps, and were found to be normal or increased in activity.

In order to determine whether endonuclease of the red cells in hereditary spherocytosis is more sensitive to fluothe than the endonuclease of normal red cells, sodium deoxyfluoride in different concentrations was added to the assay mixture and endonuclease activity was then measured. As shown in Fig. 1, under these conditions the endonuclease activity of red cells of hereditary spherocytosis was similar to that of the controls.

In conclusion, no glycolytic enzymatic deficiency was demonstrable in the erythrocytes of patients with hereditary spherocytosis. Specifically, the activity of endonuclease was normal or increased. There was no difference in the sensitivity to fluorothe of the endonuclease activity of red cells of hereditary spherocytosis as compared with normal subjects under our assay conditions.

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1 Radiation was 2,800 ergs/mm². DNA was extracted from normal L cells in logarithmic phase of growth by the technique of Zamenhof-Reiner8. Our DNA preparation contained some RNA.

Cells in the logarithmic phase of growth, suspended in saline A, were irradiated by ultra-violet light. The concentration of cells during irradiation was 0-8 × 10⁶ cells/ml. Just after radiation cells were resuspended in the appropriate medium (10 cells/ml of culture), containing highly polymerized DNA extracted from normal L cells in concentration of 20-80 μg/ml. The samples for counting cells were taken every day.

From the results depicted in Figs. 1 and 2, it is evident that highly polymerized DNA prepared from the same strain of cells aids recovery, that irradiated L cells treated with ultra-violet light root the irradiated cultures better than the control.

As can be seen from the Fig. 1 in irradiated cultures treated with 80 μg DNA/ml only 10 per cent of irradiated cells died in a 4-day interval following irradiation, whereas in non-treated culture virus as suggested for the same time-interval about 50 per cent of irradiated cells lost the capacity to divide. This result can also be seen from Table I. It seems to depend upon the amount of DNA added to the irradiated cultures. We have examined this effect with 21, 50, 60 and 80 μg of DNA/ml of culture.

Table 1. Recovery Effect of Various Amounts of DNA Added to Irradiated L Cells

<table>
<thead>
<tr>
<th>Amount of DNA added (μg/ml)</th>
<th>No. of cells surviving on the fourth day after irradiation (μg/ml)</th>
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<td>21</td>
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<td>50</td>
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<td>60</td>
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<td>80</td>
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Those results show that a significant survival has been obtained with 60 and 80 μg of DNA/ml of culture and observed results may have an important correlation with a similar DNA recovery effect in irradiated rats, observed earlier in this Laboratory.

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High-Resolution Autoradiography without Loss of Water-soluble Ions

The liquid-emulsion, stripping film, and wet-mounting autoradiographic techniques developed by Belanger and Lobeloff, Pollock and Evans, respectively, are not directly applicable to tissue which contains water-soluble radioactive material. Variations to the last two of these techniques have been described in subsequent papers. Canny9, Eisen7, and Winteringham12 described variations for mounting the stripping film without water coming into contact with the tissue, and Gallimore, Harris5, and Kominski suggested different techniques for flattening and mounting parafilm sections directly on autoradiographic plates.

The purpose of this communication is to describe a technique which provides a faster and less complex method for mounting parafilm sections directly on autoradiographic plates. By combining the procedures of flattening, mounting, and autoradiography into a single operation, this technique was devised to study the distribution of highly water-soluble sulphur-35 in barley roots.

The procedure gives good results when the parafilm is quick frozen in liquid nitrogen-cooled isopentane, and cut into pieces 2-3 mm long in a cryostat. They are then dehydrated by the freeze-dry method and indurated with a solution of methyl cellulose by Jensen13. After embedding in parafilm, sections are cut with a rotary microtome. A ribbon, containing the autoradiographic plate is then pressed against the parafilm, and the whole is placed on top of a rubber stopper of convenient size.

The subsequent operations are all performed in a well ventilated dark room using a proper safe light. For large-scale preparations these manipulations are carried out in a ventilated glove box to prevent the film becoming charged by the dark room environment.

The parafilm ribbon that has been placed on the rubber stopper is transferred to the autoradiographic plate by inverting the stopper over it. The plate is next placed on a movable base to facilitate later manipulations. Two lead 'L's' of an embedding box are arranged on the plate to form an enclosure around the autoradiographic plate. The embedding fluid used in this study were prepared by wet-mounting pieces of Kodak 'AR-10' stripping film, with the emulsion sides upward, on 'subbed' glass slides.

The arrangement of slides is inserted into a container filled with enough tolenule to cover the autoradiographic plate to a depth of 2-3 mm. The tolenule flows around the enclosure, dissolves the parafilm and leaves the cleared tissue sections on the surface of the film. After about 3 min, the arrangement is removed from the tolenule. The tolenule that is trapped within the lead enclosure is removed by gently pressing the straight edge of absorbent paper against the outside junction of the enclosure and the autoradiographic plate. Removing the tolenule in this manner permits the tissue sections to remain undisturbed on top of the film in the enclosure. The parafilm is then removed, leaving the tissue sections and the autoradiographic plate is dried in a stream of air from a fan. The plates are then stored in a light-tight box in a refrigerator for exposure.

After the desired exposure-time has elapsed, the plates are removed and prepared for photographic development according to common procedures. The plates are then washed with 1 M sodium thiosulphate for 30 sec, then dried in a stream of air. This step helps to ensure that all the tissue sections stay in contact with the autoradiographic plate during the fixing procedures. If water-soluble ions are lost from the tissue at this point, it is of no consequence since no further exposure of the film occurs. The plates are then placed for 3 min in a coplin staining dish containing tolenule to remove any trace of parafilm that might interfere with the development of the
Molecular biology in Serbia

- Serbia was one of the few countries in the world with dedicated molecular biology study direction in 1970s
- It was a top-notch study programme recognized worldwide
- While the study programme remains excellent, the main drawback are lab exercises and opportunities for practical work

• Consequence: students have excellent and broad knowledge, but very little lab experience
Personal experience

• Studied molecular biology in Belgrade
  • Diploma in 2002
• Researcher at Institute of Nuclear Sciences, Belgrade 2001 - 2006
• Research stays at Michigan State University, USA in 2004 and 2005
  • Magister of science 2005
• Research stay at University of Lübeck, Germany 2005/6
Serbs attract more Serbs

• Before my stay, the Lab in Lübeck hosted 2 molecular biologists from Serbia
• Good experience motivated the Lab head to bring more students from Serbia, and I was one of them
• After me, there were two Serbian molecular biologists visiting the Lab

B. Culjkovic → A. Djarmati → **M. Guzvic** → A. Rakovic → V. Dobricic →...
Personal experience

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  • Diploma in 2002
• Researcher at Institute of Nuclear Sciences, Belgrade 2001 - 2006
• Research stays at Michigan State University, USA in 2004 and 2005
  • Magister of science 2005
• Research stay at University of Lübeck, Germany 2005/6
• Start of PhD in Regensburg in 2006
Why Regensburg?

• I initially applied for position in Munich, but the whole Lab moved to Regensburg 6 months later
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• PhD (2006)
Why Regensburg?

• I initially applied for position in Munich, but the whole Lab moved to Regensburg 6 months later
• PhD (2006), followed by Postdoc (2011)
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Serbs attract more Serbs

Milan Obradovic
PhD
(now Basel)
Serbs attract more Serbs

Milan Obradovic
PhD
(now Basel)

Ana Grujovic
PhD
(now Telexos)
Serbs attract more Serbs

Natasa Stojanovic
PostDoc (PhD at TUM)

Milan Obradovic
PhD (now Basel)

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PhD
(now Telexos)

Natasa Stojanovic
PostDoc
(PhD at TUM)

Vladan Milosevic, MD
PostDoc
(PhD in Italy)
Others in Regensburg...

• There are/were few other Serbian molecular biologists in other Labs in Regensburg
Others in Regensburg...

• There are/were few other Serbian molecular biologists in other Labs in Regensburg

• ... and many many over the years in Munich
Currently...

• Frequently contacted by students or researchers from Serbia
  • Students want to do internships
  • Researchers wish to collaborate on projects, get expertise, or train students

• Visits possible if they obtain own maintenance funding

• Research collaboration barely existing
  • Incompatible research interests
  • Difficulties in obtaining funding for such collaborations
Recent experience....

• Early summer 2019 attempted to apply for joint German-Serbian DAAD project

• Paperwork was very complex and time-consuming
  • Hardly any German researcher would use his time for this
We need

• Money that enables mobility and collaboration
  • Travel and maintenance costs
  • Basic costs for research projects
    • Seed money for obtaining grants from major funding agencies
Conclusions/Perspective

• Serbian molecular biologists are gladly accepted in German laboratories
  • Both short-term and long-term stays
• Many would return to Serbia
• Many would not leave at all if there would be more possibilities to "plug-in" into cutting-edge research