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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) MOLECULAR AND BIOLOGICAL STUDIES ON VISUAL PROCESSES (Studies on Vision)		5. TYPE OF REPORT & PERIOD COVERED Technical
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) H. Gobind Khorana		8. CONTRACT OR GRANT NUMBER(s) NO-014-82-K-0668
9. PERFORMING ORGANIZATION NAME AND ADDRESS Massachusetts Institute of Technology Dept. of Chemistry, 18-511 77 Massachusetts Ave., Cambridge, MA 02139		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS Molecular Biology Program, Code 441MB Office of Naval Research 800 N. Quincy St., Arlington, VA 22217		12. REPORT DATE 6/15/84
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) Office of Naval Research Massachusetts Institute of Technology Rm. E19-628 Cambridge, MA 02139		15. SECURITY CLASS. (of this report)
		15a. DECLASSIFICATION DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) <div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 10px auto;">This document has been approved for public release and other distribution is unlimited.</div>		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) See attached progress report.		

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S/N 0102-LF-014-6601

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PROGRESS REPORT SUBMITTED TO THE
OFFICE OF NAVAL RESEARCH

Full Title: Molecular and Biological Studies on Visual
Processes

Abbreviated Title: Studies on Vision



Principal Investigator: H. Gobind Khorana
Alfred P. Sloan Professor
of Biology and Chemistry

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June 15, 1984

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PROGRESS REPORT

Office of Naval Research
Contract No. N00014-82-K-0668
July 1, 1983 - May 31, 1984

Submitted by H. Gobind Khorana
Massachusetts Institute of Technology
Departments of Biology and Chemistry
Cambridge, Massachusetts 02139

I. STRUCTURE FUNCTION STUDIES ON BACTERIORHODOPSIN,
THE PROTON PUMP OF HALOBACTERIUM HALOBIVM

Previously, we have reported the identification and cloning of the gene for the purpose of specific amino acid replacements. We have used three different and general methods for codon alterations at the gene level.

- (a) Point Mutagenesis: This has been carried out using gene fusion and passing the plasmids through mutator strains.
- (b) Amber Mutations and their Suppression: This has been done by using gene fusion and passing the plasmids through mutator strains.
- (c) Replacement of Restriction Fragments by Synthetic DNA Duplexes: This uses the method that we have previously developed for the chemical and enzymatic synthesis of DNA fragments.

1. The Identification of the Messenger RNA, the Nature of the Promoter Region and Initiation of Transcription

mRNA for BR has been purified by hybrid selection methods. The messenger RNA starts only two nucleotides beyond the 5' end of the structural gene. The promoter region has thus been identified.

2. Characterization of Insertion Elements that Inactivate the Bacteriorhodopsin Gene

Insertion elements have now been shown to provide a general mechanism for inactivation of the BR gene.

II. STUDIES ON BIOCHEMISTRY OF VISION

1. Bovine Rhodopsin

- (a) cDNA and genomic clone banks have been prepared. Sequence of rhodopsin cDNA corresponding to most of the structural gene has been determined.
- (b) Originally the plan was to express the rhodopsin cDNA in a suitable expression vector. Following this, we were to begin structure-function studies by changing amino acid codons. We have now undertaken the total synthesis of the structural gene. This would allow unrestricted capacity to change or delete any given regions of the rhodopsin protein.
- (c) Denaturation and Renaturation of Rhodopsin:
This study is in progress. It is fundamental to our obtaining functional or properly folded rhodopsin after expression of the gene in E.coli or other hosts.

2. Cloning and Sequencing of the GTPase and cGMP Phosphodiesterase

The cDna's for these enzymes that are involved in the amplification mechanism in the ROS are being cloned by using polyclonal antibodies against them as the probes. Antibodies that react against α , β and γ subunits have been prepared. cDNA's prepared from enriched mRNA's have been cloned into the phage λ gt11 system. Partial amino acid sequence of γ subunit has been determined.

3. Nature of the Na⁺ Channel in ROS

Work on this has just been started.

III. JUSTIFICATION FOR EQUIPMENT

Beckman Ultracentrifuge Rotors: The Beckman 50Ti rotor is one of the most highly demanded rotors in the lab. It is used at least 100 hours per month in various applications such as isolating plasmid DNA from bacterial cultures and fractionating cellular extracts. The rotor presently in use (serial no. 1803 is 14 years old, and should be replaced to avoid a potential safety hazard.

The Beckman SW28 rotor is a large-capacity swinging bucket rotor that is used for differential centrifugation of

cellular extracts, in particular, for the preparation of purple membrane from *H. Halobium* in the course of purifying bacteriorhodopsin. Formerly, there were two SW27 rotors in the lab. One of these (serial no. 1717) was retired in June, 1983 after a Beckman inspection determined that the hangers were bent and the buckets corroded, presenting a serious safety problem. The second (serial no. 4127) is out of warranty and can no longer safely be used at maximum speeds.

RC-3B Centrifuge and Rotor H-6000A: The existing centrifuges in the laboratory do not permit centrifugation of cultures larger than 300 ml at a time per bottle. Therefore, it involves repeated centrifugation involving time up to 2 hours to spin down cells from a litre of culture, especially when multiple samples are to be handled causing delay and mechanical damage to the sample. The addition of RC-3B and the rotor H-6000A would decrease the time involved to 15 min. and increase the handling capacity to 6 litres at a time. In addition, it would provide versatility to use tubes of capacity from 1-45 ml which are so frequently used in the laboratory.

H. Gobind Khorana
Contract No. N0014-82-K-0068

List of Publications

1. Bacteriorhodopsin Precursor: Characterization and Its Integration into the Purple Membrane. J.S. Seehra and H.G. Khorana, (1984) J. Biol. Chem., 259, 7, pp. 4187-4193.
2. Bacterio-opsin mRNA in Wild-type and Bacterio-opsin Deficient Halobacterium halobium Strains, S. DasSarma*, Uttam L. RajBhandary*, and H.G. Khorana*⁺, (1983) Proc. Natl. Acad. Sci. USA, 81, pp. 125-129.
3. Specific Amino Acid Substitutions in Bacterio-opsin: I. Replacement of a Restriction Fragment in the Structural Gene by Synthetic DNA Fragments Containing Altered Codons, K.-M. Lo, S.S. Jones, N.R. Hackett, and H. Gobind Khorana, (1984) Proc. Natl. Acad. Sci. USA, 81.
4. Site-Specific Mutagenesis of the Bacterior-opsin Gene: Replacement of Restriction Fragments with Synthetic DNA Duplexes Containing Altered Codons. Kin-Ming Lo*, Simon S. Jones*, Dae-Sil Lee*, Neil R. Hackett*, H. Gobind Khorana (1984) (Abstract).
5. The Cloning of the Genes for the GTPase and Cyclic GMP Phosphodiesterase of Rod Outer Segments. Kimio Yatsunami*, Bharat V. Pandya*, Daniel D. Oprian*, and H. Gobind Khorana (1984) (Abstract).

PROPOSED BUDGET

July 15, 1984 - July 14, 1985

SALARIES AND WAGES:

H. Gobind Khorana, Principal Investigator, 10%*	\$ - 0 -
One Postdoctoral Associate	20,000

EMPLOYEE BENEFITS:

39% of Salaries and Wages	7,800
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TRAVEL:

To attend scientific meetings	1,000
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MATERIALS AND SERVICES:

Chemicals, glassware, radioisotopes, lab supplies, dry ice, etc.	15,623
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EQUIPMENT:

Sorval HB4 rotor	2,000
Beckman SW28 rotor	6,045
Beckman 50Tirotor	5,680
RC-3B centrifuge and rotor H-6000A	13,095

OTHER DIRECT COSTS:

Telephone, postage, publications and xeroxing	750
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INDIRECT COSTS:

62% of the Modified Total Direct Cost Base	<u>28,007</u>
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TOTAL PROPOSED BUDGET	\$100,000
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*Dr. Khorana holds the Alfred P. Sloan Professorship in the Departments of Biology and Chemistry and his salary is paid from this source.

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