EXHIBIT 3
Part 2 of 5

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR Goldwasser, Eugene

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Eugene Goldwasser	Professor of Biochemistry	10/14/22

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
The University of Chicago, Chicago, Il.	S.B.	1943	Biochemistry
The University of Chicago, Chicago, Il.	Ph.D.	1950	Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

Positions:

Research Associate: Department of Biochemistry, The University of Chicago 1952-1961
Associate Professor of Biochemistry: The University of Chicago 1962-1963
Professor of Biochemistry, The University of Chicago 1963-present
Chairman, Committee on Developmental Biology, The University of Chicago 1976-present
Chairman, Dept of Biochemistry & Molecular Biology 1984-present

Honors:

Guggenheim Fellowship Oxford University, U.K. 1966-1967
AAAS Fellow

Publications:

Koeffler HP and Goldwasser E. Erythropoietin radioimmunoassay in evaluating patients with polycythemia. Ann. Int. Med. 94:44-47, 1981.

Weiss TL and Goldwasser E. The biological properties of endotoxin-free human erythropoietin. Biochem. J 98:17-21, 1981.

Goldwasser E. Erythropoietin and red cell differentiation in Control of Cell Division and Development. Eds. D Cunningham, E Goldwasser, D Watson and CF Fox pp 487-494, AR Liss, New York, 1981.

Goldwasser E and Sherwood JB. Radioimmunoassay of erythropoietin. Brit. J. Haematol. 98:359-364, 1981.

Tong BD and Goldwasser E. The formation of erythrocyte membrane proteins during induced differentiation. J Biol. Chem. 256:19222-12672, 1981.

Distelhorst CS, Wagner DS, Goldwasser E and Adamson JW. Autosomal dominant familiar erythrocytosis due to autonomous erythropoietin production. Blood 58:1155-1158, 1981.

Ely JM, Prystowsky MB, Eisenberg L, Quintans J, Goldwasser E, Glasebrooke AL and Fitch FW. Alloreactive cloned T cell lines. J. Immun. 127:2345-2349, 1981.

Nijhof W, Wiergena PK and Goldwasser E. The regeneration of stem cells after a bone marrow deparession induced by thiamphenicol. Exp. Hematol. 10:36-43, 1982.

Goldwasser E. Some thoughts on the nature of erythropoietin-responsive cells. J. Cell. Physiol. Suppl. 1. pp 133-137, 1982.

Weiss TL, Kavinsky C and Goldwasser E. Characterization of a monoclonal antibody to human erythropoietin. Proc. Natl. Acas. Sci. 79:5465-5469, 1982.

Shalhoub RM, Rajan U, Kim VV, Goldwasser E, Kark JA and Antoniou LD. Erythrocytosis in patients on long-term hemodialysis. Ann. Int. Med. 97:686-690, 1982.

Prystowsky MD, Ely JM, Beller DI, Eisenberg L, Goldman J, Goldman M, Goldwaaser E, Ihle J, Quintans J, Remold H, Vogel SN and Fitch FW. Alloreactive cloned T cell lines VI. Multiple lymphokine activities secreted by helper and cytolytic cloned T lymphocites. J. Immunol. 129:2337-2344, 1982.

Kawakita M, Ogawa M, Goldwasser E and Miyake T. Characterization of human megakaryocyte colony-stimulating factor in the urinary extracts from patients with aplastic anemia and idiopathic thrombocytopenic purpura. Blood, 61:556-560, 1983.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR OR AWARD CANDIDATE (Last, first, middle)	SOCIAL SECURITY NUMBER
Goldwasser, Eugene	494-14-6535

Goldwasser E. Editor, Regulation of hemoglobin synthesis: The Third Symposium of The University of Chicago Comprehensive Sickle Cell Center. Elsevier, New York, 1983.

Prystowsky MB, Ely J, Vogel SN, Goldwasser E and Fitch FW. Biochemical enrichment of lymphokines secreted by a cloned helper T lymphocyte. Fed Proc. 42:2757-2761, 1983.

Beru N, Sahr K and Goldwasser E. Inhibition of heme synthesis by succinylacetone: Effect on globin synthesis in bone marrow cells. J. Cell. Biochem. 21:93-105, 1983.

Sahr K and Goldwasser E. The effect of erythropoietin on the biosynthesis of translatable globin mRNA. in Regulation of hemoglobin biosynthesis. Ed. E. Goldwasser, Elsevier, New York, p 153-161, 1983.

Lappin TRJ, Rich I and Goldwasser E. The effect of erythropoietin and other factors on DNA synthesis by mouse spleen cells. Exp. Hematol. 11:661-666, 1983.

Wang FF and Goldwasser E. The purification of a human urinary colony-stimulating factor. J. Cell Biochem. 21:263-276, 1983.

Goldwasser E, Ihle JN, Prystowsky MD, Rich I and Van Zant G. The effect of interleukin-3 on hemopoietic precursor cells. in Symposium on Normal and Neoplastic Hematopoiesis, eds. DW Golde, and PA Marks. AR Liss, p. 301-310, 1983.

Weiss TL, Kung CKH and Goldwasser E. Erythropoietin binding to bone marrow and spleen cells. in Symposium on Normal and Neoplastic Hematopoiesis, eds. DW Golde and PA Marsk, AR Liss, p 455-464, 1983.

Prystowsky MB, Ihle JN, Otten G, Keller J, Rich I, Naujokas M, Loken M, Goldwasser E and Fitch FW. Two biological distinct colony-stimulating factors are secreted by a T lymphocyte clone. in Symposium on Normal and Neoplastic Hematopoiesis. eds. DW Golde and PA Marks, AR Liss, p. 369-378, 1983.

Prystowsky MB, Ely JM, Naujokas MF, Goldwasser E and Fitch FW. Partial purification and characterization of a colony-stimulating factor secreted by a T-lymphocyte clone. Exp. Hematol. 11:931-143, 1983.

Prystowsky MB, Naujokas MF, Ihle JN, Goldwasser E and Fitch FW. A Microassay for colony-stimulating factor based on thymidine incorporation. Amer. J. Path. 114:149-156, 1984.

Hopfer SM, Sunderman FW, Reid MC and Godlwasser E. Increased immunoreactive erythropoietin in serum and kidney extracts of rats with Ni_3S_2 induced erythrocytosis. Res. Commun. Chem. Path. Pharm. 43:299-305, 1984.

Van Zant G and Goldwasser E. Erythropoietin and its target cells. in Growth and Maturation Factors, ed. G Guroff, John Wiley, New York, 1984.

Emmanouel DS, Goldwasser E and Katz, AI. Metabolism of pure human erythropoietin in the rat. Am. J. Physiol. 247:168-176.

Krantz SB and Goldwasser E. Specific binding of erythropoietin to spleen cells infected with the anemia strain of Friend virus. Proc. Nat. Acad. Sci. in press. 1984

Goldwasser E. The characteristics and function of factors affecting erythropoiesis. Kroc Foundation Symposium, in press, 1984.

Goldwasser E, Krantz SB and Wang FF. Erythropoietin and erythroid differentiation MD Anderson Symposium, in press, 1984.

Weiss TL, Kung CKH and Goldwasser E. The frequency of bone marrow cells that bind erythropoietin. J. Cell Biochem. in press, 1984.

Sherwood JB and Goldwasser E. Erythropoietin production by human renal carcinoma cells in culture. Endocrinology, 99:504-510, 1976.

Miyake T, Kung CKH and Goldwasser E. Purification of human erythropoietin. J. Biol. Chem. 252:5558-5564, 1977.

Sherwood JB and Goldwasser E. Extraction of erythropoietin from normal kidneys, Endocrinol. 103:866-870, 1978.

Eliason JF, Van Zant G and Goldwasser E. The relationship of hemoglobin synthesi to erythroid colony and burst formation. Blood, 53:935-946, 1979.

Van Zant G and Goldwasser E. Competition between erythropoietin and colony-stimulating factor for target cells in mouse marrow. Blood, 53:946-965, 1979.

Sherwood JB and Goldwasser E. A radioimmunoassay of erythropoietin. Blood 54:885-893, 1979.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR Goldwasser, Eugene

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Fung-Fang Wang	Research Associate	5/5/48

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
National Taiwan Univ. (Taipei, Taiwan)	B.S.	1970	Agricultural chemist.
Rutgers Univ. (New Brunswick, NJ)			Biochemistry
Indiana Univ. (Bloomington, IN.)	Ph.D.	1977	Chemistry
City of Hope Med Ctr(Duarte,CA.)			Immunology
Univ. of Chicago. (Chicago, Il.)			Biochemistry

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1977-1978 City of Hope Medical Center Junior Research Scientist
Purification and characterization of fibronectin and carcino embryonic antigen.

1979-present The Univ. of Chicago Research Associate
Purification and characterization of human urinary colony stimulation factor, structure studies of erythropoietin. Binding of epo to its receptor, Partial purification and characterization of a colony stumulating factor from our embryonic kidney cell line.

PUBLICATIONS:

1. F. F. Wang and E. Goldwasser. 1983 Purification of a human urinary colony stimulating factor. J. Cell. Biochem. 21:263-275.

2. F.F. Wang and E. Goldwasser. 1983 Some chemical properties of erythropoietin. Fed. Proc. 42:1872(abstract).

3. F.K. Lin, C.H. Lin, S. Suggs, P.H. Lai, R. Smalling, J. Browne, J. Egrie, F.F. Wang and E. Goldwasser. 1984, Cloning and expression of the monkey erythropoietin gene. Fed. Proc. 43:1724.

4. F.F. Wang, C.K.H. Kung and E. Goldwasser. Some chemical properties of human erythropoietin. (submitted to Endocrinology for publication).

5. M.S. Dordal, F.F. Wang and E. Goldwasser. The role of carbohydrate in erythropoietin action. (Submitted to Endocrinology for publication).

6. E. Goldwasser, S.B. Krantz and F. F. Wang. 1984. Erythropoietin and erythroid differentiation. M.D. Anderson Symposium (in press).

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR Goldwasser, Eugene

BIOGRAPHICAL SKETCH

Give the following information for key professional personnel listed on page 2, beginning with the Principal Investigator/Program Director. Photocopy this page for each person.

NAME	TITLE	BIRTHDATE (Mo., Day, Yr.)
Phillip B. Maples	Post-doctoral trainee	6/27/56

EDUCATION (Begin with baccalaureate or other initial professional education and include postdoctoral training)

INSTITUTION AND LOCATION	DEGREE (circle highest degree)	YEAR CONFERRED	FIELD OF STUDY
University of Tulsa,	B.S.	1978	Microbiology
Graduate Coll of Med.,Oklahoma Univ Health Sciences Center	Ph.D.	1984	Biochem. & Molec. Biol

RESEARCH AND/OR PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order previous employment, experience, and honors. Include present membership on any Federal Government Public Advisory Committee. List, in chronological order, the titles and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

8/1976-6/1978 Medical Technician, Hillcrest Medical Center, Tusla Oklahoma.
Jan-May, 1978 Lab Assistant, Dept of Biology, Univ of Tulsa, Tulsa, Ok.
Jan-May, 1978 Lab Assistant, Dept of Chemistry, Univ of Tulsa, Tulsa, OK.
11,1978-6,1979. Research Technician, Dept of Biochem. & Molec. Biology, OU Health Sciences Center, Oklahoma City, OK.
7,1979-6,1982. Graduate Res. Assistant, Dept of Biochemistry and Molecular Biology OU Health Sciences Center, Oklahoma City, OK.

PUBLICATIONS

1. Broyles, R.H., G.M. Johnson, P.B. Maples and G.R. Kindell. Two erythropoietic microenvironments and two cell lines in bullfrog tad-.poles. Devel. Biol. 81: 299-314, 1981.

2. Broyles, R.H., A.R. Dorn, P.B. Maples, G.M. Johnson, G.R. Kindell and A.M. Parkinson. Choice of hemoglobin type in erythroid cells of Rana catesbeiana. in Hemoglobin in Development and Differentiation (B. Stamatoyannopoulos and A.W. Neinhuis, eds.), Alan R. Liss, Inc., New York, 1981.

3. A.M. Parkinson, A.R. Dorn, P.B. Maples and R. H. Broyles. Improved electrophoretic separation of hemoglobins by standard PAGE with different amino acid buffers. Anal. Biochem. 117:6-11, 1981.

4. P.B. Maples, A.R. Dorn and R.H. Broyles. Coexistance of embryonic and larval hemoglobins during the early development of the bullfrog Rana catesbeiana. Devel. Biol. 96:515-519, 1983.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Goldwasser, Eugene

OTHER SUPPORT

(Use continuation pages if necessary)

For each of the professionals named on page 2, list, in three separate groups: (1) active support; (2) applications and proposals pending review or funding; (3) applications and proposals planned or being prepared for submission. Include *all* Federal, non-Federal, and institutional grant and contract support. If none, state "none." For each item give the source of support, identifying number, project title, name of principal investigator/program director, time or percent of effort on the project by professional named, annual direct costs, and entire period of support. (If part of a larger project, provide the titles of both the parent project and the subproject and give the annual direct costs for each.) Describe the contents of each item listed. If any of these overlap, duplicate, or are being replaced or supplemented by the present application, delineate and justify the nature and extent of the scientific and budgetary overlaps or boundaries.

PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR:
(1) ACTIVE SUPPORT:

NIH Grant CA 18375; Hemopoietic Stem Cells and Induced Differentiation, P.I. Eugene Goldwasser (20%), direct costs 05/01/84 to 04/30/85, $80,364, period of support 07/01/78 to 06/30/88.

NIH Grant HL 30121; Program Project, The Biology of Sickle Cell Disease, P.I. Eugene Goldwasser (10%), 04/10/84 to 03/31/85; Sub Project VI, Study of the Regulation of Hemoglobin Synthesis in Bone Marrow Cell. Direct costs $57,406, period of support 04/01/83 to 03/31/88.

2. Pending
This application - 09 yr HL 21676

3. Planned
None

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR: Goldwasser, Eugene

RESOURCES AND ENVIRONMENT

FACILITIES: Mark the facilities to be used at the applicant organization and briefly indicate their capacities, pertinent capabilities, relative proximity and extent of availability to the project. Use "other" to describe the facilities at any other performance sites listed in Item 9, page 1, and at sites for field studies. Using continuation pages if necessary, include an explanation of any consortium arrangements with other organizations.

[X] Laboratory: Approx. 1500 sq. ft: fully operating, including culture labs, and needed equipment, and a cold room.

[XX] Clinical: When needed, the Clinical Research Center can be made available. It can be used for any further clinical testing.

[XXX] Animal: Carlson Animal Research Facility is used to house all lab animals and to maintain them in a healthy state for experimental purposes.

[XXX] Computer: A micro computer with hard copy and graphics output.

[XXX] Office: There are separate offices for the P.I. and the secretary.

[ ] Other (_____):

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

HPLC, Gas chromatograph, culture hoods, incubators, centrifuges, monitors β and γ counters, spectrophotometers are all within the lab.

ADDITIONAL INFORMATION: Provide any other information describing the environment for the project. Identify support services such as consultants, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.

Secretarial service within the lab; the machine shop is an important adjunct as noted in the budget justification.

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PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR OR AWARD CANDIDATE (Last, first, middle)	SOCIAL SECURITY NUMBER
Goldwasser, Eugene	494-14-6535

SPECIFIC AIMS

Among the various polypeptide cellular growth factors that have been under study in recent years, erythropoietin (epo) occupies a special position. Its existence has, probably, been known longer than any other growth factor, yet much less is known about its chemistry and mode of action. This is due, clearly, to the very limited quantities of pure epo available. Another special aspect of epo biology relates to its high degree of specificity; unlike many of the other growth factors, the result of epo action is the formation of a single class of differentiated blood cells, erythrocytes.

We propose, here, to continue our intensive study of both the chemistry and biology of epo and to continue, as well, the extension of our laboratory work to possible clinical applications. More specifically, we plan to devote considerable effort to the study of the structure of epo in order to understand the chemical basis of its specific biological activity. We especially want to study the structure of the active region (or regions) and the relationship between that structure and its interaction with specific cellular receptors of epo. Simultaneous with these studies we plan to continue to work on the possibility that smaller and simpler fragments of epo may be biologically active. Both of these kinds of study, necessarily will involve further investigation of the epo receptor and we plan to extend our work in this field in two directions: the further chemical characterization of the receptor from virus-infected cells and the study of normal cell receptors.

We plan to use our newly developed tools, such as the monoclonal anti-epo and the cloned epo DNA, to study the regulation of epo biogenesis in normal kidney and/or fetal liver cells. In addition, the current availability of a line of mouse cells that constitutively secretes substantial amounts of epo into the medium, will make it possible to study the path of biosynthesis of this glycoprotein.

We plan to also extend our studies of epo levels in disease states, but will first concentrate on improving the current RIA by using the monoclonal anti-epo, developed in this lab, in solid state assay which will rely on the antibody for specificity rather than on pure epo.

Lastly, we will continue our several collaborations with other laboratories in the study of both clinical and experimental aspects of epo biology.

SIGNIFICANCE

The central role of epo in the normal regulation of mammalian red cell formation is now well established, as well as its importance in a wide array of fields such as, clinical hematology, experimental hematology, cell differentiation, hormone action and eukaryotic gene expression. Study of the biochemistry and molecular biology of epo and of its molecular and cellular modes of action are now more timely than ever.

These are several aspects of the research planned in this proposal; some of these are now under study in this laboratory and we propose to continue with them. The subjects we are now investigating or plan to study and the rationale for each follow:

Purification Until the mass production of biologically active epo, based on recombinant DNA is accomplished, there will be a real need for "natural" epo from urine, plasma or culture media. Since all of these sources are limited, improved purification methods will be needed; improved especially with respect to yield. We proprose to continue our work[1] on developing a rapid, simple and high yielding method which will, in addition, be applicable to epo produced by recombinant methods as well.

Structure Because of the striking specificity of epo action in the induction of red cell formation, it is of general importance with respect to cell differentiation to understand the detailed mechanism of how this particular glycoprotein exerts its effect. Ultimately the action of epo on its target cells must be a function of its structure and the structure of its receptor. There are several aspects to the structure of a glycoprotein that should be studied: the primary structure of the polypeptide. the primary structures of the oligosaccharide chains and the secondary and tertiary structures of the holoprotein. Our working hypothesis is that there is a region of the polypeptide that is less tightly structured than the remainder and that interacts

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with a specific epo receptor on responsive cells. The conformation of this active region may be dependent, in part, on the secondary and tertiary structures which may be defined by the relationship between the hydrophilic oligosaccharides and the generally hydrophobic protein.

We have shown that immunoreactive material, smaller in molecular size than native epo, (termed "fragments" for convenience) can be found in the sera of some patients with chronic renal disease.[2] We propose to isolate and characterize these fragments with the idea that one or more may contain the active region of epo and may interact with receptors on hemopoietic precursor cells and thus block epo action. If this proves to be the case we may find an explanation for some of the anemias of chronic renal disease and perhaps other anemias. In addition, there remains the possibility that a fragment smaller than native epo may be biologically active.

Cloning of epo Now that the human epo gene has been cloned[3,4] and work is underway to produce epo commercially in large quantity, we plan to use similar methods to obtain cloned epo DNA from mouse, rat and rabbit to compare sequences and to study common structural features that may be important in biological activity. Probes derived from this area of research will be useful in study of biogenesis as outlined below.

Biosynthesis of epo The isolation of IW32 cells (a mouse line)[5] that make substantial quantities of epo in culture now makes it possible to study the path of its biosynthesis. We plan to examine the questions of whether epo is produced as a larger precursor, whether there are regions of the putative precursor that are required for transmembrane passage leading to secretion and what mechanisms regulate glycosylation of the protein. Since the secretion of up to 1U/ml appears to be constitutive, we plan to determine whether increased secretion by these cells can be affected by addition of substances known to have an effect in vivo. If so, we will be able, then, to study the mechanism of regulation of epo secretion and/or biosynthesis. This problem has not been able to be studied rigorously in the past. The question of how expression of the epo gene in these cells, and others, is regulated will also be studied using a specific nucleotide probe capable of hybridizing with epo mRNA.

Radioimmunoassay Investigation of many problems in epo biochemistry and physiology requires a rapid, specific and highly sensitive assay method. None of the assay systems available at present meets all of these requirements. We propose to study the development of a solid-state immunoassay, based on the monoclonal anti-epo developed in this laboratory with the requisite sensitivity and speed of analysis.

Epo receptor studies Now that we have shown the existence of specific receptors for epo in Friend cells (anemic variant, FVA)[6], we plan to extend this work in two directions, the purification and characterization of the FVA mouse receptors and the extension of receptor studies to normal erythropoietic cells. These problems are closely connected to our need to know the mechanism by which epo exerts its effect on target cells. One key problem in the study of cell differentiation, in general, and of red cell formation in particular, lies in the interaction between inducer (ligand) and sensitive cell. The question of whether the cellular program, resulting in massive hemoglobin synthesis, is set in motion by internalized epo-receptor complexes or whether by a trans-membrane signal, not involving internalization, cannot be answered without detailed knowledge of the specific receptor and its properties.

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