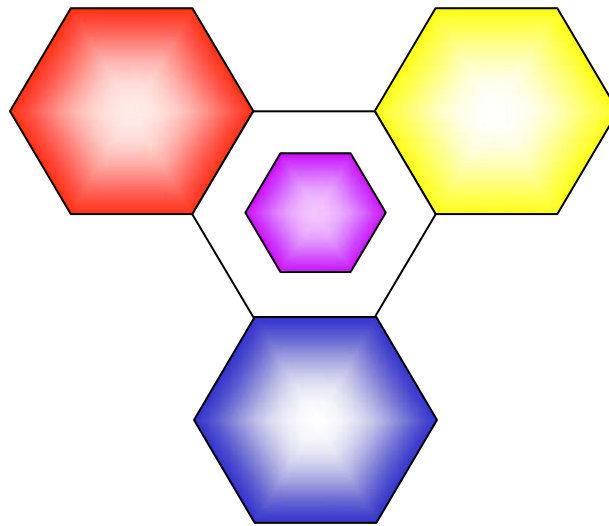


# Improving your chances to obtain a Vici grant

*a personal view*



John van der Oost

# Research project – *find the balance*

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- innovative
- feasible
- track record



# Research proposal – *convincing & clear*



- title & abstract
- idea & approach
- management

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**NWO**  
Vici scheme

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**Registration form (basic details)**

**1a. Details of applicant**

-Name, title(s): John van der Oost, Dr.  
-Male/female: Male  
-Address for correspondence: Laboratory of Microbiology, Wageningen University  
Hesselink van Suchtelenweg 4, 6703 CT Wageningen, NL  
-Preference for English correspondence: no  
-Telephone: 0317-483108  
-Fax: 0317-483829  
-E-mail: john.vanderoost@wur.nl  
-Website (optional): <http://www.fms.wau.nl/mkr/>  
-Doctorate (date): 21-06-1989  
-Use of extension clause (see Notes): no

**1b. Title of research proposal**

**Back to the future – unravelling eukaryal-like control networks in archaea**

**1c. Summary of research proposal**

The evolution of life has resulted in three fundamentally distinct classes of organisms: the eukaryotic domain and two prokaryotic domains, the bacteria and the more recently discovered archaea. Comparative genomics and biochemical analyses have indicated that the prokaryotic archaea and the eukarya are closely related with respect to cellular information processing systems, such as replication, transcription, translation, as well as protein modification and turnover. A set of 55 proteins has been found to be completely conserved in archaea and eukarya, but is not encoded by any of the bacterial genomes. Whereas the majority of this archaeal-eukaryal core of proteins has been demonstrated to play an important role in information processing, a significant number of these well-conserved proteins has not yet been subjected to detailed analysis, neither in archaea nor in eukarya. Recent studies in the applicant's group have suggested that one of these proteins, the Multi-Protein Bridging Factor (MBF), is a so-called "master global regulator" of the archaeal cell. It was observed that MBF interacts with archaeal-eukaryal core proteins that have potential regulatory functions, and include basal transcription factors, ribosomal proteins and a proteasome regulatory-subunit.

This project aims at the identification and functional characterization of global regulatory networks that control the modulation of the archaeal cell's proteome composition, by regulation at the level of transcription, translation and proteolysis. In our working hypothesis, master global regulators (such as MBF) interact with specific regulatory components of the respective information processing machineries. *Sulfolobus solfataricus* is a thermophilic archaeon that serves as a model organism since its complete genome sequence, genetic systems, and functional genomics tools have recently been established by active participation of the applicant's group. Potential regulatory networks will be analysed (i) by monitoring global phenotypes (transcriptome, proteome) in *S. solfataricus* wild-type and to-be-generated mutant strains (silenced or enhanced expression of selected target genes), (ii) by identifying *in vivo* interactions of potential regulators with proteins and DNA fragments, and (iii) by *in vitro* functional characterization of selected regulatory proteins and their target proteins and/or DNA. Ultimately, this study should contribute to unravelling the global regulation network in archaea, gain insight in the mechanism of archaeal signal transduction, and provide details on the evolution of the well-conserved archaeal-eukaryal information processing systems.

**Keywords:** Archaea, Evolution, Transcription, Translation, Proteasome

**1d. NWO Council area**  
ALW (Aard- en levenswetenschappen; Geo/Life sciences)

**1e. Host institution**  
Laboratory of Microbiology, Wageningen University, the Netherlands

# Research proposal – *convincing & clear*



- title & abstract
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adjusting functionality. Jacob & Monod (1961, 1963) pioneered with the regulation at transcription level, and also provided the first evidence for allosteric regulation of proteins. Specific regulation of the flow from gene to protein can occur at all possible levels in the information processing from DNA to Protein (Fig. 2): (i) DNA - transient modification that affects local transcription activity (binding of histone (-like) proteins; methylation); (ii) DNA/RNA - transcription regulation by specific and global regulatory proteins (activation and repression, by single- and multiple-component regulatory proteins), (iii) RNA - transient modification that affect its translation (binding of proteins, small RNAs and metabolites to mRNA); (iv) RNA/Protein - translation regulation by specific interactions that affect ribosome functionality (e.g. stringent response in bacteria); and (v) Protein - transient modification (e.g. assisted folding by chaperones, complex formation with partner proteins, allosteric regulation by metabolites, modifications such as phosphorylation, methylation, acetylation and ubiquitinylation). Another important level of functional regulation concerns the turnover of mRNA and protein, by RNases (degradosome complex in bacteria; exosome complex in archaea and eukarya, see below) and proteases (ClpXP in bacteria, proteasome in archaea and eukarya; see below).

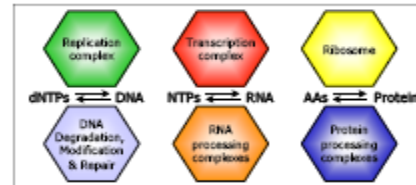


Figure 2. Control Levels. Cellular control is determined by the balance of production versus degradation of the key molecules in Information Processing. Adjusting at the level of the genome (DNA) generally proceeds at a moderate pace (epigenetic modification by histones and methylation). Manipulation at transcription (RNA) and proteome (protein) level has a moderate to very fast response, because of its indirect and direct impact on functionality, respectively. See text for details.

### Discovery of the Archaea – a missing link in the evolution of the Eukaryotic Cell

Permanent modifications at DNA level are the result of (i) imperfect replication of the genome before cell division (point mutations during "vertical gene transfer"), (ii) damage of biochemical (DNA binding agents) or biophysical (UV light) origin, and (iii) recombination via the exchange of genetic material from parent genomes (sexual recombination), or via the exchange DNA fragments with any organism ("horizontal gene transfer"). As originally predicted by Charles Darwin (1859), the evolutionary drift of genetic information (DNA sequences) is the basis for the diversity of life forms. Classification of life has initially been performed on the basis of morphology, later in combination with biochemistry. Until three decades ago, life was divided into two domains: the prokarya and the eukarya (reviewed by Stanier and Van Niel 1962). At first glance, the morphology of organisms that belong to the prokarya domain is very similar, apparently lacking the complex composition of eukaryotic cells. Because of their morphological similarity, the co-existence of two fundamentally different types of prokaryotes was not recognized before the introduction of molecular classification techniques in the 1970s. Comparison of ubiquitous sequences such as the building blocks of the protein synthesis machinery (ribosomal RNA and protein) were used to compose universal phylogenetic trees. By introducing molecular analyses to establish phylogenetic relations, a surprising discovery was made: the obtained phylogenetic tree (Fig. 3) strongly suggested that early in the cellular evolution two domains diverged within the prokaryotes: the archaeobacteria and eubacteria. It was concluded that life should be divided into three distinct types of living systems (Woese and Fox, 1977). To stress their distinct nature, the initial names of the prokaryotic domains were later adjusted to archaea and bacteria (Woese *et al.* 1990).




Figure 3. The Tree of Life. The unrooted phylogenetic tree is based on sequence alignments of RNAs and proteins that are involved in information processing (Replication, Transcription and Translation). Adapted from Woese *et al.* 1990.

# Research proposal – *convincing & clear*



- title & abstract
- idea
- management



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*in vitro*

Table 1. Time Table of sub-projects - Work Packages (WPs) & Tasks

| WP nr   | Team member | Task nr | Name of WP/Task   | Year | 1 | 2 | 3 | 4 | 5 |
|---|-------------|---------|---|------|---|---|---|---|---|
| <b>1. Comparative Genomics</b>                      |             |         |   |      |   |   |   |   |   |
| 1.  | PI + PD     | 1.1     | Identification of potential regulators                    |      |   |   |   |   |   |
|   |             | 1.2     | Function prediction of selected genes                     |      |   |   |   |   |   |
|   |             | 1.3     | Prediction of target operon/inducers of selected genes    |      |   |   |   |   |   |
|   |             | 1.4     | Function prediction of exp. identified genes              |      |   |   |   |   |   |
| <b>2. Molecular Genetics – <i>E. coli</i></b>       |             |         |   |      |   |   |   |   |   |
| 2.  | T + PI      | 2.1     | Cloning & expr. of predicted regulators in <i>E. coli</i> |      |   |   |   |   |   |
|   |             | 2.2     | Purification and char. of predicted regulators            |      |   |   |   |   |   |
|   |             | 2.3     | Cloning & expression of tagged factors in <i>E. coli</i>  |      |   |   |   |   |   |
|   |             | 2.4     | Purification and characterization of isolated factors     |      |   |   |   |   |   |
|   |             | 2.5     | Writing articles  |      |   |   |   |   |   |
| <b>3. Functional Genomics</b>                       |             |         |   |      |   |   |   |   |   |
| 3.  | AO-1        | 3.1     | Substrate fermentation                                    |      |   |   |   |   |   |
|   |             | 3.2     | Shift experiments   |      |   |   |   |   |   |
|   |             | 3.3     | DNA microarrays - shifts                                  |      |   |   |   |   |   |
|   |             | 3.4     | DNA microarrays - mutants                                 |      |   |   |   |   |   |
|   |             | 3.5     | Writing articles and thesis                               |      |   |   |   |   |   |
| <b>4. Molecular Genetics – <i>S. sofatarius</i></b> |             |         |   |      |   |   |   |   |   |
| 4.  | AO-2        | 4.1     | Cultivation of <i>Sofatarius</i> fermenter                |      |   |   |   |   |   |
|   |             | 4.2     | Generate constructs for overproduction & knockouts        |      |   |   |   |   |   |
|   |             | 4.3     | Transformation of <i>Sofatarius</i>                       |      |   |   |   |   |   |
|   |             | 4.4     | Proteomics  |      |   |   |   |   |   |
|   |             | 4.5     | Writing articles and thesis                               |      |   |   |   |   |   |
| <b>5. Biochemistry</b>                              |             |         |   |      |   |   |   |   |   |
| 5.  | PD+T        | 5.1     | GST-pull down   |      |   |   |   |   |   |
|   |             | 5.2     | Yeast 2-hybrid  |      |   |   |   |   |   |
|   |             | 5.3     | TAP-tagging   |      |   |   |   |   |   |
|   |             | 5.4     | ChIP  |      |   |   |   |   |   |
|   |             | 5.5     | Writing articles  |      |   |   |   |   |   |
| <b>6. Data &amp; Project Management</b>             |             |         |   |      |   |   |   |   |   |
| 6.  | PI+PD       | 6.1     | Establish Data-Warehouse                                  |      |   |   |   |   |   |
|   |             | 6.2     | Import & Integrate Data                                   |      |   |   |   |   |   |
|   |             | 6.3     | Coordination & Management                                 |      |   |   |   |   |   |


characterization of different regulatory factors, the produced proteins will be used for generating antibodies that will be crucial for immunoprecipitation (incl. ChIP and ChIP-on-chip) experiments of WP 5. A PhD student will be appointed in the first year of the project, with the goal to use DNA microarray analysis to get insight in the modulation of gene expression in response to different stress situations, and in to-be-generated *S. sofatarius* mutants (WP4). A second PhD student will start in the beginning of the second year, in a sub-project on the application of the recently developed molecular genetics tools of *S. sofatarius* (WP4). The overproduction of TAP-tagged proteins has high priority, to be used for biochemical analysis of complex formation and target proteins (WP5). In addition, the disruption of genes that potentially encode global regulators will be a main goal, to be analysed at the proteome level (WP4), as well as at the transcriptome level (WP3). The main task of the Postdoc(s) will be to apply a range a biochemical analyses (WP5), most of which have been used before in different projects within the applicant's group (including GST pull-down assays, yeast 2-hybrid screening). *In vitro* Protein-Protein analyses will be performed with the recombinant proteins (with or without tags/fusions) from WP2. Based on the specific antibodies (spin-off of WP2), different types of Immunoprecipitations will be performed, including the ChIP-like methods to get insight in

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# Research proposal – *convincing & clear*



- title & abstract
- idea & approach
- management

  
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**Cost estimates**


**3a. Budget**

| costs (k€)       | raise / fte | 2005       | 2006       | 2007       | 2008       | 2009       | total       |
|------------------|-------------|------------|------------|------------|------------|------------|-------------|
| <b>Staff</b>     |             |            |            |            |            |            |             |
| Applicant        | 70% / 0.6   | 63         | 67         | 70         | 75         | 80         | 355         |
| Postdoc          | 35% / 1.0   | 48         | 51         | 56         | 60         | 64         | 279         |
| PhD-1            | 35% / 1.0   | 30         | 37         | 41         | 45         |            | 153         |
| PhD-2            | 35% / 1.0   |            | 31         | 38         | 42         | 46         | 157         |
| Technician       | 35% / 0.2   | 7          | 8          | 8          | 9          | 9          | 41          |
| <b>Non-staff</b> |             |            |            |            |            |            |             |
| Equipment        |             | 50         |            |            |            |            | 50          |
| Consumables      |             | 30         | 30         | 30         | 30         | 30         | 150         |
| Travel           |             | 10         | 10         | 10         | 10         | 10         | 50          |
| Other            |             | 15         |            |            |            |            | 15          |
| <b>TOTAL</b>     |             | <b>253</b> | <b>234</b> | <b>253</b> | <b>271</b> | <b>239</b> | <b>1250</b> |

# CV – convincing & clear



- awards
- international experience
- publications



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103. Andrew, H., Glynn, S.E., Barynin, V., Baker, P.J., Sofelnikova, S.E., Verhees, C., De Guss, D., Van der Oost, J., Tinson, D.J., Reese, R.J. and Rice, D.W. (2004) Substrate specificity and mechanism from the Structure of *Pyrococcus furiosus* galactokinase. *J. Mol. Biol.* 337, 387-398

104. Kim, J.S., Kluskens, L.D., De Vos, W.M., Huber, R., Van der Oost, J. (2004) Crystal structure of feruloylase from *Ferulobacterium jensenii* var. *ferulobacterium*, a keratinolytic enzyme related to subtilisin. *J. Mol. Biol.* 335, 787-797

**Impact Factors**

List of International Scientific Journals in which articles of the applicant have been published with Impact Factor >5. In the list of the ISI Citation Index, 70 articles by the applicant are included, with more than 700 citations.  
(ISI Web of Knowledge, Journal Citation Reports 2002; <http://isi4.isiknowledge.com>)

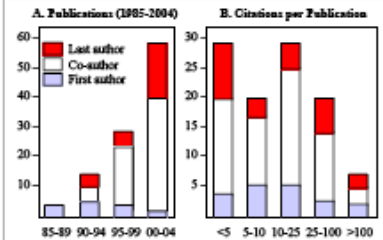
| Journal              | Impact Factors (2002) | nr. |
|----------------------|-----------------------|-----|
| Nature               | 32.4                  | 1   |
| Trends Biochem. Sci. | 14.4                  | 1   |
| Trends Genetics      | 13.2                  | 1   |
| PNAS                 | 10.7                  | 1   |
| EMBO J.              | 10.7                  | 2   |
| EMBO Reports         | 7.6                   | 1   |
| Nucl. Acid Res.      | 7.0                   | 1   |
| J. Biol. Chem.       | 6.7                   | 12  |
| Trends Microbiol.    | 6.6                   | 1   |
| J. Am. Chem. Soc.    | 6.2                   | 2   |
| Mol. Microbiol.      | 5.8                   | 7   |
| J. Mol. Biol.        | 5.4                   | 5   |

Articles with Impact Factor > 5.0: 35

**Citation Analysis**

A total of 2499 citations were reported of the total of 104 publications (on average 23.8 citations per publication (August 2004); <http://isi4.isiknowledge.com>)

(A) Number of publications in 4 periods of 5 year;  
(B) Number of publications, ranked by citation per paper (not corrected for self-citation).



**-Non-refereed journals**

Van der Oost, J. (2002) Snelle selectie in de reageerbuis. *Natuur en Techniek* 70 (5), 66-68

Van der Oost, J. (2002) Sels in de reageerbuis. *Natuur en Techniek* 70 (5), 11

Van der Oost, J. (2004) Unique features of archaical metabolism. *The Biochemist* (in press, June 2004)

**-Books, or contributions to books**

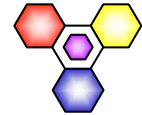
Krab, K., Peters, F.A.L.J., Van der Oost, J., de Wit, H.J., Othmann, L.F. (1984) Mediation of electron transfer between photosynthetic and hydrogen-evolving parts of biophotolytic systems. In: *Innovations in Biotechnology* (Eds. Houwink, E.H. and Van der Meer, R.R.) pp. 507-516

Van der Oost, J. (1989) *The hydrogen metabolism of the unicellular cyanobacterium Cyanosyce PCC 7822*. Ph.D. Thesis, Vrije Universiteit, Amsterdam


Smidt, H., Song, D., Van der Oost, J., De Vos, W.M. (1997) Molecular characterization of halorespiration. In: *International Symposium Environmental Biotechnology* (Ostende, Belgium) (eds. Verachert H., Verstraete

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# Referees – a lottery ?



- keywords
- reviews
- provide names ?

  
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**Registration form (basic details)**

**1a. Details of applicant**

-Name, title(s): John van der Oost, Dr.  
-Male/female: Male  
-Address for correspondence: Laboratory of Microbiology, Wageningen University  
Hesselink van Suchtelenweg 4, 6703 CT Wageningen, NL  
-Preference for English correspondence: no  
-Telephone: 0317-483108  
-Fax: 0317-483829  
-E-mail: john.vanderoot@wur.nl  
-Website (optional): <http://www.fms.wau.nl/mkr/>  
-Doctorate (date): 21-06-1989  
-Use of extension clause (see Notes): no

**1b. Title of research proposal**  
**Back to the future – unravelling eukaryal-like control networks in archaea**

**1c. Summary of research proposal**  
The evolution of life has resulted in three fundamentally distinct classes of organisms: the eukaryotic domain and two prokaryotic domains, the bacteria and the more recently discovered archaea. Comparative genomics and biochemical analyses have indicated that the prokaryotic archaea and the eukarya are closely related with respect to cellular information processing systems, such as replication, transcription, translation, as well as protein modification and turnover. A set of 55 proteins has been found to be completely conserved in archaea and eukarya, but is not encoded by any of the bacterial genomes. Whereas the majority of this archaeal-eukaryal core of proteins has been demonstrated to play an important role in information processing, a significant number of these well-conserved proteins has not yet been subjected to detailed analysis, neither in archaea nor in eukarya. Recent studies in the applicant's group have suggested that one of these proteins, the Multi-Protein Bridging Factor (MBF), is a so-called "master global regulator" of the archaeal cell. It was observed that MBF interacts with archaeal-eukaryal core proteins that have potential regulatory functions, and include basal transcription factors, ribosomal proteins and a proteasome regulatory-subunit.  
This project aims at the identification and functional characterization of global regulatory networks that control the modulation of the archaeal cell's proteome composition, by regulation at the level of transcription, translation and proteolysis. In our working hypothesis, master global regulators (such as MBF) interact with specific regulatory components of the respective information processing machineries. *Sulfolobus solfataricus* is a thermophilic archaeon that serves as a model organism since its complete genome sequence, genetic systems, and functional genomics tools have recently been established by active participation of the applicant's group. Potential regulatory networks will be analysed (i) by monitoring global phenotypes (transcriptome, proteome) in *S. solfataricus* wild-type and to-be-generated mutant strains (silenced or enhanced expression of selected target genes), (ii) by identifying *in vivo* interactions of potential regulators with proteins and DNA fragments, and (iii) by *in vitro* functional characterization of selected regulatory proteins and their target proteins and/or DNA. Ultimately, this study should contribute to unravelling the global regulation network in archaea, gain insight in the mechanism of archaeal signal transduction, and provide details on the evolution of the well-conserved archaeal-eukaryal information processing systems.

**Keywords:** Archaea, Evolution, Transcription, Translation, Proteasome

**1d. NWO Council area**  
ALW (Aard- en levenswetenschappen; Geo/Life sciences)

**1e. Host institution**  
Laboratory of Microbiology, Wageningen University, the Netherlands

# Interview – *presentation*



- handouts
- clear slides
- 1 slide/min

|          |          |          |          |           |
|----------|----------|----------|----------|-----------|
| <p>1</p> | <p>2</p> | <p>3</p> | <p>4</p> | <p>5</p>  |
| <p>6</p> | <p>7</p> | <p>8</p> | <p>9</p> | <p>10</p> |

# Interview – *discussion*



- referees' comments
- SWOT analysis
- practise



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| SWOT analysis – <i>Applicant &amp; Referees</i> |  |
|---|--|
| Strengths                                       | relatively simple, stable & sophisticated control systems of poorly studied archaena   |
| Weaknesses                                      | unprecedented methods (e.g. knock-outs), however, distinct independent research lines  |
| Opportunities                                   | experience to perform systems approach, insight in evolution of key regulatory systems |
| Threats   | international competition, however, proposed team should provide critical mass         |

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