Research Report Labor Dr. Wolfgang Marwan

Universität Freiburg
Institut für Biologie III
Schänzlestrasse 1
D-79104 Freiburg i. Br.
Germany

Sensory Control of Sporulation in *Physarum polycephalum:* Exploring a Molecular Network that Regulates Cell Differentiation

Team: Sreedhar Balaji, Petra Kramer, Marie-Luise Siegler

Projects

- Time-Resolved Somatic Complementation Analysis of Mutants
- Isolation and Functional Analysis of Early Developmentally Regulated Genes

The development of multicellular and many unicellular organisms depends on a controlled program of cell differentiation. Many differentiation processes are subjected to sensory control by external stimuli originating from their micro- or macro-environment. Thereby, animals, plants and microorganisms are able to adapt to a continually changing environment and to respond to stress or insult. These responses are mediated and controled by intelligent regulatory networks made up by the funcional interaction of proteins and other biomolecules. We have developed a new experimental approach to the analysis of the structure and function of such molecular networks: Time-resolved somatic complementation analysis is based on the fusion of mutant cells at different states of activation and subsequent cytoplasmic mixing. As exprimental system we are using plasmodia (giant single cells) of the lower eukaryote *Physarum polycephalum* which spontanesously fuse with each other upon physical contact.

Plasmodia sense visible light, starvation and heat shock through specific receptors (e.g. phytochrome and cryptochrome) and integrate them by a branched signal transduction pathway which finally controls the developmental decision to sporulate (Fig. 1). In plasmodia committed to sporulation, morphogenesis starts at about nine hours after induction, proceeds through a sequence of distinct morphogenetic intermediates and finally results in the formation of fruiting bodies that contain ripe, haploid spores (Fig. 1, lower part).

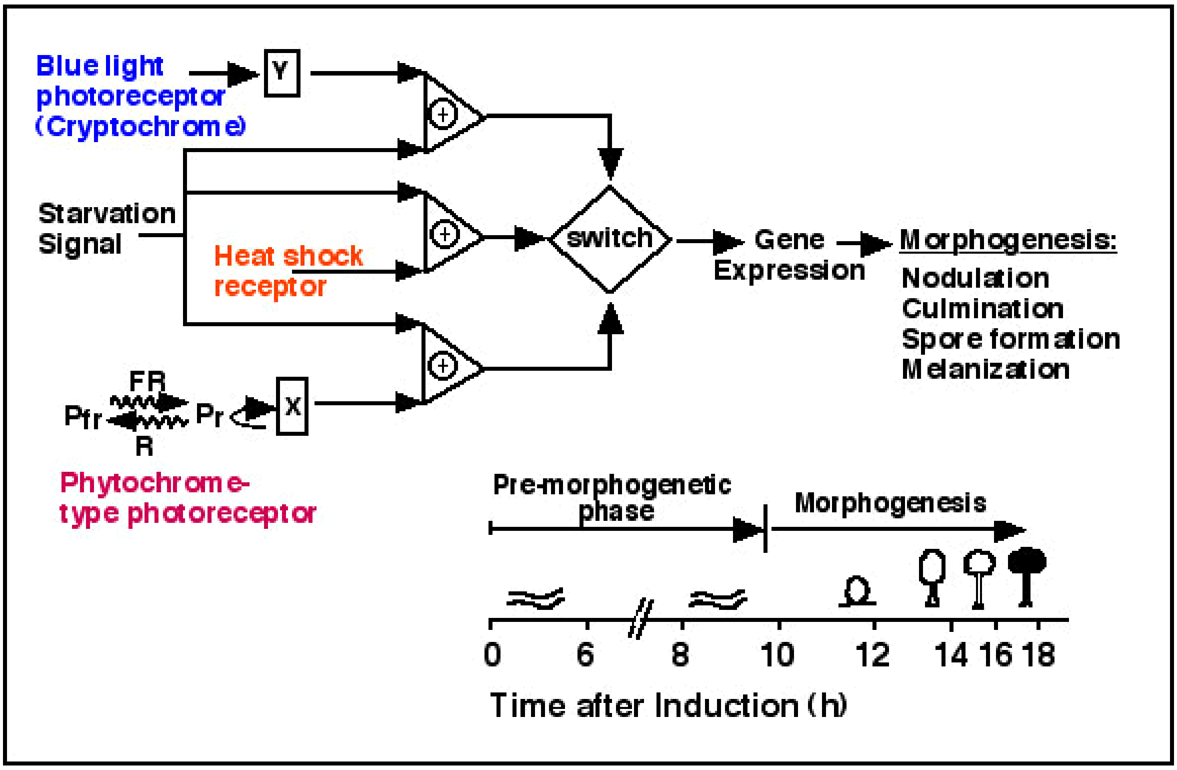

Fig. 1: Sensory control of sporulation in *Physarum polycephalum* plasmodia.

A. Time-Resolved Somatic Complementation Analysis of Mutants

Mutants defective in certain steps of the pathway mediating sensory control of sporulation were isolated and are analyzed by time-resolved somatic complementation. In a typical experiment, the signaling pathway is activated in one plasmodium and allowed to proceed for a defined period of time. The induced plasmodium is then fused to a second, genetically different plasmodium that has not been induced (Fig. 2). Whether the two plasmodia are able to complement their defects in sporulation may strongly depend on the state of activation of the signaling pathway and hence on the period of time elapsed between receptor activation and cytoplasmic mixing. This effect allows the quantitative and time-resolved detection of the activity of signaling intermediates that determine the cellular commitment to sporulation. By time-resolved somatic complementation analysis, mutants can be easily characterized and those selected for molecular analysis that display a well-defined regulatory function within the network.

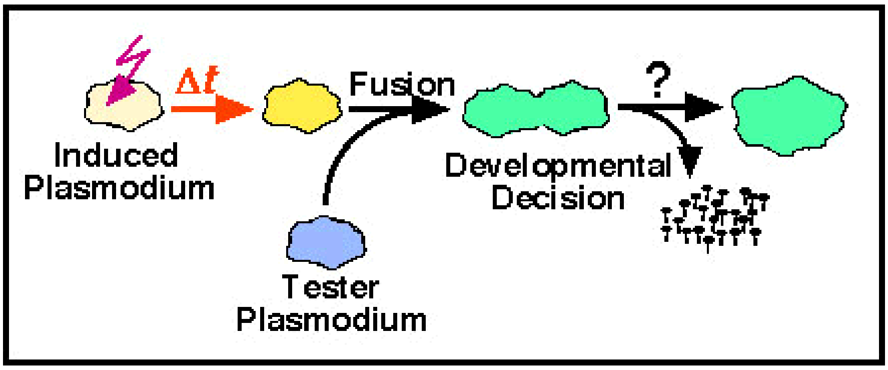

Fig. 2: Time-resolved somatic complementation analysis of sporulation-deficient mutants by plasmodial fusion.

B. Isolation and Functional Analysis of Early Developmentally Regulated Genes

Sporulation is driven by a cascade of differentially regulated genes that encode structural proteins and other factors required for fruiting body formation which starts at about 11 hours after induction (see Fig. 1, lower part). By differential display RT-PCR have found that few early genes are differentially regulated already at six hours after induction. At this time the plasmodial cell is irreversibly committed to sporulation. These early genes are interesting because their regulatory elements define targets in the central part of the network and because they might encode regulators of downstream processes.

The expression of one of these genes, *LIG1* , is associated with the developmental switch from the vegetative stage to sporulation and its expression level correlates with the probability of the plasmodial cell to sporulate. LIG1 is homologous to HUS1, a cell cycle regulator in fission yeast and to proteins from mouse and human. We think that LIG1 may be involved in the coordination of the developmental decision to sporulate with the cell cylce control during morphogenesis.

Acknowledgements

This work is supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

Publications (1999-1994, Last update: 28. 1. 2000)

- Marwan, W. 2000. Photomovement and photomorphogenesis in *Physarum polycephalum* : Targeting of cytoskeleton and gene expression by light. *In* D.-P. Häder and M. Lebert (ed.), Photomovements. Elsevier, Amsterdam, in press.
- Marwan, W., and D. Oesterhelt. 2000. Archaeal vision and bacterial smelling: Sensory control of the swimming behavior by two component signaling and fumarate. ASM News, in press.
- Marwan, W. 1999. Kryptochrome und LOV/PAS-Domänen-Proteine - vielseitige Regulatoren des Zellgeschehens. Biospektrum 5:443-448.
- Joanidopoulos, K. D., and W. Marwan. 1999. Neural integration of chemosensory and mechanosensory stimuli triggeres the mating response in males of the giant rotifer *Asplanchna sieboldi* . Ethology. 105:465-475.

- Kroneder, R., A. R. Cashmore, and W. Marwan. 1999. Phytochrome-induced expression of *lig1* , a homologue of the fission yeast cell cycle checkpoint gene *hus1* is associated with the developmental switch in *Physarum polycephalum* plasmodia. Curr. Genet. 36:86-93.

- Marwan, W., and D. Oesterhelt. 1999. Die Phototaxis der Halobakterien: Biochemische Mechanismen einer einfachen Verhaltensreaktion. Chemie in unserer Zeit. 33:140-151.

- Joanidopoulos, K. D., and W. Marwan. 1998. Specific behavioural responses triggered by identified mechanosensory receptor cells in the apical field of the giant rotifer *Asplanchna sieboldi* . J. Exp. Biol. 201:169-177.

- Marwan, W. 1998. Kommunikation ohne Worte, p. 100-103, Chemie heute, vol. 1998/99.

- Montrone, M., M. Eisenbach, D. Oesterhelt, and W. Marwan. 1998. Regulation of switching frequency and bias of the bacterial flagellar motor by CheY and fumarate. J. Bacteriol. 180:3375-3380.

- Starostzik, C., and W. Marwan. 1998. Kinetic analysis of a signal transduction pathway by time-resolved somatic complementation of mutants. J. Exp. Biol. 201:1991-1999.

- Marwan, W., and C. Starostzik. 1997. Somatische Komplementationsanalyse: ein neuer Schlüssel zum Verständnis zellulärer Regulationsprozesse? Biospektrum. 3:25-27.

- Montrone, M., D. Oesterhelt, and W. Marwan. 1996. Phosphorylation-independent bacterial chemoresponses correlate with changes in the cytoplasmic level of fumarate. J. Bacteriol. 178:6882-6887.
- Marwan, W., S. I. Bibikov, M. Montrone, and D. Oesterhelt. 1995. Mechanism of photosensory adaptation in *Halobacterium salinarium* . J. Mol. Biol. 246:493-499.

- Oesterhelt, D., and W. Marwan. 1995. Wie Archaea sehen und schmecken. Biospektrum. 1:11-16.

- Starostzik, C., and W. Marwan. 1995. Functional mapping of the branched signal transduction pathway that controls sporulation in *Physarum polycephalum* . Photochem. Photobiol. 62:930-933.

- Starostzik, C., and W. Marwan. 1995. A photoreceptor with characteristics of phytochrome triggers sporulation in the true slime mould *Physarum polycephalum* . FEBS Lett. 370:146-148.

- Krah, M., W. Marwan, and D. Oesterhelt. 1994. A cytoplasmic domain is required for the functional interaction of SRI and HtrI in archaeal signal transduction. FEBS Lett. 353:301-304.

- Krah, M., W. Marwan, A. Verméglio, and D. Oesterhelt. 1994. Phototaxis of *Halobacterium salinarium* requires a signalling complex of sensory rhodopsin I and its methyl-accepting transducer HtrI. EMBO J. 13:2150-2155.

- Kupper, J., W. Marwan, D. Typke, H. Grünberg, U. Uwer, M. Gluch, and D. Oesterhelt. 1994. The flagellar bundle of *Halobacterium salinarium* is inserted into a distinct polar cap structure. J. Bacteriol. 176:5184-5187.

- Starostzik, C., and W. Marwan. 1994. Time-resolved detection of three intracellular signals controlling photomorphogenesis in *Physarum polycephalum* . J. Bacteriol. 176:5541-5543.

Publications (1993-1977)

- Bibikov, S. I., R. N. Grishanin, A. D. Kaulen, W. Marwan, D. Oesterhelt, and V. P. Skulachev. 1993. Bacteriorhodopsin is involved in halobacterial photoreception. Proc. Natl. Acad. Sci. USA . 90:9446-9450.

- Ferrando-May, E., M. Krah, W. Marwan, and D. Oesterhelt. 1993. The methyl-accepting transducer protein HtrI is functionally associated with the photoreceptor sensory rhodopsin I in the archaeon *Halobacterium salinarium* . EMBO J. 12:2999-3005.

- Michal, G. 1993. Biochemical Pathways, 3 rd ed. Boehringer Mannheim GmbH, Mannheim. Montrone, M., W. Marwan, H. Grünberg, S. Mußeleck, C. Starostzik, and D. Oesterhelt. 1993. Sensory rhodopsin-controlled release of the switch factor fumarate in *Halobacterium salinarium* . Mol. Microbiol. 10:1077-1085.

- Oesterhelt, D., and W. Marwan. 1993. Signal transduction in halobacteria, p. 173-187. *In* M. Kates, D. J. Kushner, and A. T. Matheson (ed.), The biochemistry of Archaea (Archaebacteria), vol. 26. Elsevier Science Publishers, Amsterdam.

- Bibikov, S. I., R. N. Grishanin, A. D. Kaulen, W. Marwan, D. Oesterhelt, and V. P. Skulachev. 1992. The role of bacteriorhodopsin in photoreception in *Halobacterium halobium* (RS). Bioorg. Khimia. 18:1403-1423.
- Marwan, W., M. Montrone, and D. Oesterhelt. 1992. Signal transduction in *Halobacterium halobium* mediated by the switch factor fumarate, p. 313-316. *In* J. L. Rigaud (ed.), Structures and functions of retinal proteins, vol. 221. J. Libbey Eurotext Ltd.
- Marwan, W., and D. Oesterhelt. 1992. Realm of the senses. Nature. 357:654.
- Wagner, G., and W. Marwan. 1992. Locomotion, p. 126-152. *In* H.-D. Behnke, K. Esser, K. Kubitzki, M. Runge, and H. Ziegler (ed.), Progress in Botany, vol. 53. Springer Verlag, Berlin.
- Bibikov, S. I., R. N. Grishanin, W. Marwan, D. Oesterhelt, and V. P. Skulachev. 1991. The proton pump bacteriorhodopsin is a photoreceptor for signal transduction in *Halobacterium halobium*. FEBS Lett. 295:223-226.
- Marwan, W., M. Alam, and D. Oesterhelt. 1991. Rotation and switching of the flagellar motor assembly in *Halobacterium halobium*. J. Bacteriol. 173:1971-1977.
- Marwan, W., and D. Oesterhelt. 1991. Light-induced release of the switch factor during photophobic responses of *Halobacterium halobium*. Naturwiss. 78:127-129.
- Marwan, W., and D. Oesterhelt. 1990. Quantitation of photochromism of sensory rhodopsin-I by computerized tracking of *Halobacterium halobium* cells. J. Mol. Biol. 215:277-285.
- Marwan, W., W. Schäfer, and D. Oesterhelt. 1990. Signal transduction in *Halobacterium* depends on fumarate. EMBO J. 9:355-362.
- Oesterhelt, D., and W. Marwan. 1990. Signal transduction in *Halobacterium halobium*, p. 219-239. *In* J. P. Armitage and J. M. Lackie (ed.), Biology of the chemotactic response, vol. 46. Cambridge University Press, Cambridge.
- Oesterhelt, D., and W. Marwan. 1989. Signal transduction in halobacteria, p. 282-301. *In* A. E. Evangelopoulos (ed.), Receptors membrane transport and signal transduction, vol. H 29. Springer-Verlag, Berlin.
- Otomo, J., W. Marwan, D. Oesterhelt, H. Desel, and R. Uhl. 1989. Biosynthesis of the two halobacterial light sensors P480 and SR and variation in gain of their signal transduction chains. J. Bacteriol. 171:2155-2159.
- Hegemann, P., and W. Marwan. 1988. Single photons are sufficient to trigger movement responses in *Chlamydomonas reinhardtii.* Photochem. Photobiol. 48:99-106.
- Marwan, W., P. Hegemann, and D. Oesterhelt. 1988. Single photon detection in an archaebacterium. J. Mol. Biol. 199:663-664.
- Marwan, W., M. Alam, and D. Oesterhelt. 1987. Die Geißelbewegung halophiler Bakterien. Naturwiss. 74:585-591.
- Marwan, W., and D. Oesterhelt. 1987. Signal formation in the halobacterial photophobic response mediated by a fourth retinal protein (P 480). J. Mol. Biol. 195:333-342.
- Oesterhelt, D., and W. Marwan. 1987. Change of membrane potential is not a component of the photophobic transduction chain in *Halobacterium halobium*. J. Bacteriol. 169:3515-3520.
- Kraml, M., and W. Marwan. 1983. Photomovement responses of the heterotrichous ciliate *Blepharisma japonicum*. Photochem. Photobiol. 37:313-319.
- Marwan, W. 1981. Massenkulturen von Mikroorganismen in Dialysierschläuchen. Mikrokosmos. 70:213-214.
- Marwan, W. 1980. Untersuchungen über das Pigment des Wimpertieres *Blepharisma japonicum*. Mikrokosmos. 69:208-211.
- Marwan, W. 1978. Eine bemerkenswerte Art der Zellteilung beim Wimpertier *Blepharisma japonicum*. Mikrokosmos. 67:340-345.
- Marwan, W. 1977. Schwanzbildungen beim Riesenwimpertier *Blepharisma japonicum*. Mikrokosmos. 66:357-360.