Myxobacteria exhibit a social life cycle with a variety of multicellular patterns. The rod-shaped cells glide along their long axis. Vegetative cells prey, grow and divide as individuals or in small swarms. Individual-based models of cohort migration have been reviewed in [1]. Under starvation conditions, bacteria start to act cooperatively, aggregate and finally build a multicellular structure, the fruiting body. Fruiting body formation is often preceded by a periodic pattern called rippling [2-5] (Fig. 1a). Bacteria organize into equally spaced ridges (dark regions) that are separated by regions with low cell density (light regions). We examine the temporal dynamics of the density profile along a line perpendicular to the ripples (white line in Fig. 1a). The resulting space-time plot reveals a periodically oscillating standing wave pattern (Fig. 1b) with a wavelength of about 10-20 cell length and a temporal period of 10 min. Single cells move unidirectionally with the ripple waves in a typical back-and-forth manner [4].

Intercellular communication is essential to maintain a complex life cycle as the one exhibited by Myxobacteria. While other bacteria and amoebae (e.g. Dictyostelium discoideum) signal via diffusible chemicals (chemotaxis), myxobacterial rippling apparently depends on the membrane-bound protein C-factor; here signalling requires close end-to-end contact of cells [4]. Addition of C-factor (which can be extracted from rippling cells) increases the mean reversal frequency of cells [4]. Mutants that carry a mutation in the C-factor-encoding gene, are unable to ripple and aggregate. Experiments with dilutions of C-signal-competent cells such mutants exhibit an increased ripple wavelength [4]. Based on these findings Sager and Kaiser proposed the following mechanism for ripple formation: When two opposite moving cells collide head-on, they reverse their gliding direction due to exchange of C-factor [4]. In order to test this hypothesis we have designed a mathematical model.

Figure 1: (a) Ripples in aggregates of myxobacteria (snapshot from a movie by H.Reichenbach). The grayscale expresses to the cell density. White bar: 300 µm. (b) Standing wave pattern in a space-time plot of the density along the white bar in (a) (parallel to the direction of wave propagation).
**Discrete model.** - Our model for the formation of ripple patterns is based on the dynamics of individual cells. It is defined on a regular cubic lattice assuming discrete space and time coordinates, analogous to cellular automaton models. The spatial lattice constants are chosen in a way that bacterial cells (assumed as equally sized) cover exactly one node. Allowed cell positions are (i) directly on the substrate or (ii) on top of other cells. This reflects the experimental situation in which cells glide on the surface and are organized in heaps [3]. The total number of cells is constant (absence of replication and death). Once per time step all cells move to the neighboring node in accordance to their orientation. Several exception scenarios ensure mobility of the densely packed cells. The basic interaction rule of the model is derived from the hypothesis described in the previous section. In the model, head-on-collision takes place if two counter-moving cells form a spatial arrangement in a way that their leading ends are close enough. A cell can be invoked in more than one collision event. Furthermore we assume cells to be either sensitive or refractory. Refractory cells do not respond to C-factor, i.e. only sensitive cells reverse if invoked in collisions. After a cell has reversed it is temporarily refractory. The duration of this refractory phase is the most important ingredient for rippling [6]. We have also investigated the effect of introducing a second cell type representing C-factor deficient mutants. These mutants fail to encode the C-factor protein, thus they do not induce reversal of other cells in collisions. The mutant cell itself can receive C-signals and reverses after collisions with non-mutant cells. Reversal of mutants also results in temporary refactororiness; here we assume equal duration of this phase for mutants and non-mutants. There are no transitions between the mutant and the non-mutant cell type.

**Mean-field approximation.** - Apart from direct simulation one can perform a linear stability analysis on the following deterministic rate equations [6]:

$$
\begin{align*}
    r_1(x, t+1) &= r_1(x-1, t) - F_r(x-1, t) + r_r(x-1, t) \\
    r_2(x, t+1) &= F_l(x-1, t) \\
    r_i(x, t+1) &= r_{i-1}(x-1, t), \\
    l_1(x, t+1) &= l_1(x+1, t) - F_r(x+1, t) + l_r(x+1, t) \\
    l_2(x, t+1) &= F_l(x+1, t) \\
    l_i(x, t+1) &= l_{i-1}(x+1, t), \quad i = 3 \ldots \tau.
\end{align*}
$$

$l_i$ and $r_i$ denote the average density of left- and right-moving sensitive ($i = 1$) and refractory ($i = 2 \ldots \tau$) cells. The reversal functions $F_r(x, t)$ and $F_l(x, t)$ estimate the collision probabilities.

**Simulation results and discussion.** - The refractory period turns out to be crucial for ripple formation. Regular patterns for refractory times $\tau \geq \tau_c \approx 4 \text{ min}$ can be observed. Two counter-propagating travelling waves of about equal amplitude form a standing wave, in agreement with the experimental observations. For refractory times $\tau < \tau_c$ we still observe pieces of waves without long-range correlations in space or time; for vanishing refractory time cells exhibit fluctuations near a homogeneous density state (Fig. 2a-f). Wavelength and wave period of the simulated ripples increase with $\tau$ [6]. The experimental values of wavelength and temporal period of the macroscopic rippling pattern (Fig. 1) are reproduced with a refractory time of ca. 4.5 min. The homogeneous solution of the rate equations (1) becomes linear unstable against an oscillatory instability for $\tau \geq 4 \text{ min}$ (Fig. 3a). The comparison of wavelength and period of the most unstable
Figure 2: Simulation snapshots for several refractory times ((a) \( \tau = 1 \) min, (b) \( \tau = 3 \) min, (c) \( \tau = 5 \) min). In (d-f) we show the corresponding space-time-plots. Please note the similarities of (c),(f) and Fig.1.

Figure 3: (a) Numerically obtained real part of eigenvalues of the linearization of equation (1) with a suitably chosen reversal function [6] for sub- and supercritical values of \( \tau \). Only the branch with the rippling instability is shown. We compare wavelength (b) and period (c) of the ripple pattern found in simulations (squares) and in the mean-field equations (lines).

mode with the equivalent quantities extracted from Fourier analysis of the simulated data shows good agreement, in particular for \( \tau < \tau_c \) (Figs. 3b,c).

The cell-based nature of the model enables us to investigate also the behavior of individual cells. They are found to move about a distance of half a wavelength before reversing [6], reproducing the back-and-forth movement of cells in the experiment. Patterns produced in mutant-diluted systems (model extension I) are shown in Fig. 4. For a fairly large fraction (40%) of C-signal-defective mutants (Fig. 4a,d) the pattern is still recognizable and regular with long correlation lengths in space and time. Upon further increase of mutants the patterns are no longer visible from a snapshot (Figs. 4b,c), though the space-time diagrams still show some periodicity and wave propagation (Figs.4 e,f). In the simulations the wavelength of the ripples does not show any clear tendency to change with the mutant fraction; this does not agree with the reported experiments [4].

**Conclusions.** - We presented a simple individual-based model for ripple formation including the interplay between cell migration and orientation-dependent interaction rather than a reaction-diffusion mechanism. The spatio-temporal synchronization is due to a refractory phase after reversal during which further cell reversal is prohibited. As our
results show, collision-induced cell reversal as proposed by Sager and Kaiser is an appropriate mechanism for ripple formation only if it is supplemented by a refractory period. Wavelength and period of the pattern are determined by the duration of this period. The emergence of the pattern sensitively depends on the precision of this internal clock. Myxobacterial rippling provides a first example of pattern formation mediated by migration and direct cell-cell interaction. A similar mechanism may also be involved in myxobacterial fruiting body formation as well as selforganization processes in other multicellular systems.


