Nocodazole, Vinblastine and Taxol at Low Concentrations Affect Fibroblast Locomotion and Saltatory Movements of Organelles

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Microtubules (MTs) are essential for the maintenance of asymmetric cell shape and motility of fibroblasts. MTs are considered to function as rails for organelle transport to the leading edge. We investigated the relationship between the motility of Vero fibroblasts and saltatory movements of particles in their lamella. Fibroblasts extended their leading edges into the experimental wound at a rate of 20 ±11 µm/h. Intracellular particles in the front parts of the polarized fibroblasts moved saltatorily mainly along the long axis of the cells. MT depolymerization induced by the nocodazole at a high concentration (1.7 µM) resulted in the inhibition of both fibroblast motility and saltatory movements of the particles. Taxol (1 µM) inhibited the fibroblast locomotion but not the saltatory movements. The saltatory movement pattern was disorganized by taxol by decreasing the portion of longitudinal saltations and consequently by increasing the part of saltations perpendicular to the cell long axis. This effect may be explained by disorganization of the MT network resulting from the inhibition of dynamic instability. To further investigate the relationships between the MT dynamics instability, saltatory movements, and fibroblast locomotion, we treated fibroblasts with microtubule drugs at low concentration (nocodazole, 170 nM; vinblastine, 50 nM; and taxol, 50 nM). All these drugs induced rapid disorganization of the saltatory movements and decreased the rate of cell locomotion. Simultaneously, the amount of acetylated (stable) MTs increased. The treatment also induced reversible changes in the actin meshwork. We suggest that decrease in the fibroblast locomotion rate in the case of MT stabilization occurred because of the appearance of numerous free MTs. Saltations along free MTs are poorly organized and, as a result, the number of organelles reaching the fibroblast leading edge decreases.

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Fibroblasts in *vitro* are polarized cells that move over glass. A moving fibroblast exhibits several morphologically different cytoplasmic regions—these are lamellipodia, lamella, the cell body with a nucleus, and the tail [1]. Two stages may be differentiated in the movement of fibroblasts: (1) advancement of the lamella and its fixation on a substrate; (2) pulling-up of the cell body and tail. The leading edge of the fibroblast is the margin where the lamellipodia are situated. It is important to note that in the moving fibroblast all the edges of the cytoplasm, except the lamellipodia, are stable. The fibroblast polarization is maintained by the cytoskeleton: actin and microtubules (MTs). Under the action of colcemid, which disassembles MTs, the actively undulating membrane appears all over the free fibroblast edge; however, the cell movement is blocked [2]. Thus, the disassembly of MTs perturbs the polarization of the cell and suppresses its movement.

The basic function of MTs in animal cells is intracellular transport of different organelles. Organelles move along the MTs with the aid of the motor proteins dynein (towards the minus end of MT) or kinesin (towards the plus end of MT) using the ATP energy. Such movements are of jump-type and are called saltatory [3, 4]. This is the manner of movement, for example, of endosomes and lysosomes [5], mitochondria [6], as well as of other organelles.

One explanation of the MT role in the movement of fibroblasts may be the organization of the transfer of membrane vesicles from the Golgi apparatus to the cell leading edge. Vesicles may be incorporated there into the cell membrane, and this may result in the increase of the membrane area and growth of lamellipodia in a strictly definite direction. There are numerous data supporting this hypothesis. In the first place, an intact MT system is necessary for restricting the zone of actively undulating membrane in a rather narrow region of the cell surface (of lamellipodia) [2, 7]. Secondly, upon inhibition of kinesin, providing for the transport of organelles along MTs towards the fibroblast leading edge, the lamellipodial activity is suppressed and the elongated bipolar shape of the fibroblast is lost [8]. Thirdly, the movements of granules in the fibroblast lamella visible under a light microscope occur mostly in two directions: from the nucleus to the fibroblast leading edge and back from its leading edge to the nucleus [9].

It is widely believed that the MTs, like rails, organize a directed movement of organelles. However, the MTs are highly labile structures, too. The ends of individual MTs in the cell continuously grow and become shorter. Such an alternation of phases was termed dynamic instability of MTs [10]. The dynamic instability of MTs in living cells may be suppressed by low concentrations (-100 nM) of mitostatics (e.g., nocodazole, vinblastine or taxol) when they do not yet modify appreciably the equilibrium between MTs and the pool of soluble tubulin [11-14]. Low concentrations of nocodazole
and taxol have been shown recently to decelerate nearly two times the movement of NRK fibroblasts into a wound [15].

Hence, the following question arises: how the dynamic instability of MTs is related to their transport function in the cell? An attempt to answer this question can be made by studying the effects resulting from the suppression of dynamic instability of MTs.

The aim of our study was to compare changes in the locomotion of fibroblasts of Vero line over the substrate with variations of parameters of saltatory movements of intracellular granules that take place in these cells upon suppression of the dynamic instability of MTs by different substances.

**EXPERIMENTAL**

**Cell culture.** Our studies were performed using a culture of Vero line cells (fibroblasts from the African green monkey kidney) from the collection of cell cultures of the Institute of Cytology (Russian Academy of Sciences). The cells were cultured at 37°C in 5% CO₂ in a mixture of DMEM and F12 media (1:1) (Sigma, USA) with 3% fetal calf serum. Gentamicin (100 µg/ml) was used as an antibiotic.

**Vital observations.** For experiments, cells were placed onto round coverslips. For vital observations, coverslips with cells were mounted into an original chamber for studies of living cells [16]. The present study used a model of experimental wound described in detail previously [9]. In 2 h after the removal of a part of the monolayer with a razor blade, coverslips were mounted into the chamber. Vital observations were conducted immediately after the mounting, their duration did not exceed 4 h. In the course of observations the temperature on the microscope stage was maintained at 37°C with a fan (Nicolson Precision Instruments, USA). Video recording was performed using a high-sensitive matrix telecamera with a resolution of 500 TV lines and an AG-6730 video tape recorder (Panasonic, Japan). To decrease the photolesion of cells, orange and green luminophores were employed for video recording. To analyze the migration of cells into the wound and the saltatory movements of intracellular organelles, the cells localized at the edge of the wound and having a clear-cut polarity were selected. Migration and saltatory movements of cells were analyzed in analogous cells but in different experiments.

Analysis of the migration of cells from the monolayer into free space of the wound relied on continuous video recording of 6-10 wound edge-based cells for 4 h with the use of a high-speed mode of the video tape recorder with a 63-fold acceleration. A Neofluar lens (16/0.40) was employed, and the final magnification on the monitor screen was x 1000.

Saltatory movements of intracellular granules were analyzed in the fibroblast lamella. To this end, video recording was performed in the real time
mode. Each cell was video recorded for 5 min. A Planapo lens (63/1.4) was employed, and the final magnification on the monitor screen was x6300.

**Mitostatics.** Experiments were performed using the following mitostatics: nocodazole (Sigma) at concentrations of 1.7 µM and 170 nM, vinblastine (Sigma) at a concentration of 50 nM and taxol (Sigma) at concentrations of 1 µM and 50 nM. Video recordings of the same cells prior to the mitostatic addition were used as controls. In one instance, to analyze the cell migration into the wound, the movement of cells was continuously recorded for 2 h and then, without disassembling the chamber, a mitostatic was added and the video recording was continued for another 2 h. In another case, to analyze the saltatory movements, 5-min-long video recording of two selected cells was performed. Then the initial medium was replaced by a medium containing a mitostatic, and after this, 5-min-long video recordings of the same cells were performed in 10, 30, 60, 90, and 120 min.

An analogous experiment in a mitostatic-free medium was used as control.

**Analysis of data.** To analyze the mean rate of the advance of the leading edge of cells from the monolayer into the wound, only strictly polarized cells were used in which the leading edge advanced in the control through the observation period. Our preliminary experiments showed that in one and the same cell the propagation of its leading edge occurred at a constant rate during a 4-h observation period. The mean rate of the advance of the leading edge of cells was calculated in the following manner. For each cell, we determined the distance to which the point of its leading edge the most distant from the nucleus center moved for every 30 min. Then the data on the mean rates of propagation of different cells were averaged.

Analysis of saltatory movements was carried out in the following manner. Translocations of individual granules were continually observed on the monitor screen and drawn with a felt-tip pen on a transparent film laid on the screen. The number of tracks and their lengths were determined and a sample of angles between tracks (or their extensions) and the long axis of the lamella was analyzed. The long axis of the lamella is a straight line passing through the nucleus center and the middle of the segment connecting the widest points of the lamella (Fig. 1a). The long axis did not change its position through the 5-min video recording period. However, in each 5-min video recording the long axis in the same cell was built anew. The tracks directed towards the nucleus center were assigned positive values of angles, whereas the tracks going from the nucleus center were assumed to form negative angles. Thus, the entire sample of angles was within the range from -90 to 90° and included only integer values. The data obtained were statistically processed using the program Statistica for Windows. All the data are presented with indication of their standard deviations.

**Immunofluorescence.** Double staining of cells with phalloidin (Sigma) and antibodies to α-tubulin (Sigma) was carried out to reveal the active
cytoskeleton and the MT system. To detect the acetylated MTs, we used monoclonal murine antibodies C3B9 to acetylated tubulin, which was the kind gift of Prof. K. Gull (University of Manchester, Great Britain). Before staining, the cells were washed with Hanks' solution (pH 7.4) at 37°C, then fixed with 2.5% glutaraldehyde for 5 min, washed 3 times with phosphate buffered saline (PBS, pH 7.2-7.4), and treated with NaBH₄ (2 mg/ml) twice for 10 min. Then the cells were permeabilized for 20 min in acetone at -20°C. Finally, staining with antibodies was performed using an indirect technique.
In the case of double staining, the cells were first stained with phalloidin conjugated with FITC and then with the TAT antibodies to α-tubulin, which were kindly provided by Prof. K. Gull (University of Manchester, Great Britain), and the secondary antibodies conjugated with Texas Red. After staining the cells were additionally fixed with 2.5% glutaraldehyde for 20 min. The preparations obtained were placed into glycerol containing 1,4-diazobicyclo[2,2,2]octane (DABCO, Sigma Chemical Co., USA) and examined under an Opton-3 photomicroscope (Germany). The preparations were photographed using an RF-3 film (Tasma, Russia).

RESULTS

The objects of our studies were the fibroblasts localized at the edge of the experimental wound and possessing large well spread lamellae. Such fibroblasts migrated from the monolayer into the experimental wound. The fibroblast migration from the monolayer into the wound is a complex process consisting of the lamella advancement and pulling-up of the cell body and tail. According to preliminary data, the mean rate of the cell leading edge propagation corresponded to the speed of the entire cell movement (data not shown). To simplify analysis of the results obtained, we concentrated solely on the process of lamella advancement. The mean speed of advancement was constant through the entire observation period (from 2 to 6 h after the wound placement on the monolayer) and was equal to 20±11 µm/h (156 cells).

Intensive saltatory movements of spherical granules were observed to occur in the lamella of fibroblasts polarized at the wound edge. On the average, the cell lamella was found to contain about 50 spherical granules from 0.3 to 1.4 µm in diameter. Over 50% of them were represented by lipid droplets [9]. Lysosomes were absent in the lamella and were found only in the perinuclear zone of the cell body [9].

Within 5 min, 134±49 saltatory movements of granules occurred on the average in the lamella of a single cell. The mean length of granule tracks was 6.0±2.0 µm (21 cells, n = 3076). The maximum track length was 30 µm. Saltatory movements of granules in the lamella occurred in two main directions: from the nucleus to the cell leading edge and back - from the cell leading edge to the nucleus. The tracks of granules were mostly parallel to the long axis of the lamella, i.e., they were axially arranged (Fig. 1a). In this case, the tracks that were directed towards the cell leading edge exhibited a slight prevalence (56±5%). The tracks perpendicular to the long axis of the cell were very rare.

To describe quantitatively the disposition of tracks in the lamella, the angles formed by the tracks and the long axis of the lamella were measured. The mean angle of the tracks with the axis was close to zero (1°, n = 3076). The standard deviation from the mean value was 21°.


When any directions of the granule movement are equally probable, the mean angle between tracks and any chosen axis would be 0°, while the standard deviation is 52°. The smaller standard deviation of tracks in the lamella indicates that the directions of tracks relative to the lamella long axis were not equally probable. Indeed, the distribution of angles formed by the tracks and the long axis of the lamella displayed a clear-cut peak corresponding to the mean value of the sample (Fig. 1 b). We called such a
Figure 2. The cytoskeleton in Vero cells, a, b, c - control; d, e, f - 10 min after addition of nocodazole (170 nM); g, h, i - 120 min after nocodazole addition (170 nM). The upper and middle series of photographs - double staining of the same cell for actin (FITC-phalloidin) and immunofluorescent staining for tubulin, respectively. The bottom series of photographs - immunofluorescent staining of another cell for acetylated tubulin. Scale bar, 20 µm.

distribution of the tracks of saltatory movements in a polarized cell "ordered", and it can be satisfactorily described by the law of Gaussian distribution.

The overwhelming majority of MTs in the lamella were parallel, or nearly parallel, to the long axis of the lamella (Fig. 2b). Stable (acetylated) microtubules in cells were extremely rare (Fig. 2c). The acetylated microtubules were mostly localized in the perinuclear region.
As a rule, there were nearly no stress-fibrils in the lamella of Vero fibro-blasts (Fig. 1d). When the stress-fibrils were found in this cytoplasm zone, their quantity did not exceed 10 and they were mostly parallel to the long axis of the lamella and its distal zone and were distributed more chaotic in the proximal zone.
Total disassembly of MTs suppresses translocation of fibroblasts and saltatory movements of granules. In order to check whether the dislocation of cells and saltatory movements of granules in them are dependent on the presence of MTs, nocodazole was applied at a concentration of 1.7 \( \mu \text{M} \). Immediately after addition of nocodazole the migration of fibroblasts into the wound was blocked. During the first hour after the addition of nocodazole, the cells drew inside the lamellipodia by 10 \( \mu \text{m} \). Then the cells started to release lamellipodia again. The lamellipodia protruded to the distance up to 10 \( \mu \text{m} \). But since each such protrusion of lamellipodia was followed by their retraction to nearly the same distance, no summary translocation of cells took place.

Incubation of fibroblasts with nocodazole decreased rapidly the quantity of saltatory movements of granules (Fig. 3a, Table 1) and reduced the mean length of tracks (Table 1). In 2 h after nocodazole application, virtually all the saltatory movements were blocked.

Table 1. Alterations of the parameters of saltatory movements of granules under the action of different mitostatics.

<table>
<thead>
<tr>
<th>Mitostatic</th>
<th>Number of tracks within 5 min ± S.D.</th>
<th>Track length ± S.D, ( \mu \text{m} )</th>
<th>% of tracks from the nucleus ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nocodazole, 1.7 ( \mu \text{M} )</td>
<td>128±70</td>
<td>5.79±1.78</td>
<td>56±5</td>
</tr>
<tr>
<td>0</td>
<td>63±35</td>
<td>4.46±1.21</td>
<td>44±10</td>
</tr>
<tr>
<td>10</td>
<td>22±7*</td>
<td>4.05±1.32*</td>
<td>34±3*</td>
</tr>
<tr>
<td>60</td>
<td>8±2*</td>
<td>3.43±1.10*</td>
<td>-</td>
</tr>
<tr>
<td>90</td>
<td>6±5*</td>
<td>3.09±1.19*</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>1±1*</td>
<td>2.76±0.73*</td>
<td>-</td>
</tr>
<tr>
<td>Taxol, 1 ( \mu \text{M} )</td>
<td>242±62</td>
<td>5.58±2.06</td>
<td>52±12</td>
</tr>
<tr>
<td>0</td>
<td>159±37</td>
<td>4.87±1.56*</td>
<td>48±7</td>
</tr>
<tr>
<td>10</td>
<td>143±52</td>
<td>4.69±2.12*</td>
<td>43±6</td>
</tr>
<tr>
<td>30</td>
<td>129±42*</td>
<td>4.29±1.12*</td>
<td>43±3</td>
</tr>
<tr>
<td>60</td>
<td>123±31*</td>
<td>4.09±0.99*</td>
<td>36±19</td>
</tr>
<tr>
<td>90</td>
<td>127±46*</td>
<td>4.09±0.88*</td>
<td>41±3</td>
</tr>
<tr>
<td>120</td>
<td>161±75</td>
<td>5.15±1.52</td>
<td>55±6</td>
</tr>
<tr>
<td>Nocodazole, 170 nM</td>
<td>126±73</td>
<td>4.73±1.35</td>
<td>53±7</td>
</tr>
<tr>
<td>0</td>
<td>113±70</td>
<td>3.86±0.93</td>
<td>50±7</td>
</tr>
<tr>
<td>10</td>
<td>86±47</td>
<td>3.92±1.02</td>
<td>55±2</td>
</tr>
<tr>
<td>60</td>
<td>90±47</td>
<td>4.29±0.93</td>
<td>45±18</td>
</tr>
<tr>
<td>90</td>
<td>81±37</td>
<td>4.35±0.98</td>
<td>54±5</td>
</tr>
</tbody>
</table>
Table 1. Alterations of the parameters of saltatory movements of granules under the action of different mitostatics.

<table>
<thead>
<tr>
<th>Mitostatic</th>
<th>Number of tracks within 5 min ± S.D.</th>
<th>Track length ± S.D, µm</th>
<th>% of tracks from the nucleus ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vinblastine, 50 nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>129±69</td>
<td>4.97±1.61</td>
<td>54±7</td>
</tr>
<tr>
<td>10</td>
<td>85 ±66</td>
<td>4.60±1.52</td>
<td>38±10*</td>
</tr>
<tr>
<td>30</td>
<td>44±13*</td>
<td>4.44±1.86</td>
<td>34±12*</td>
</tr>
<tr>
<td>60</td>
<td>27±14*</td>
<td>3.53±0.89*</td>
<td>31±7*</td>
</tr>
<tr>
<td>90</td>
<td>22±6*</td>
<td>3.99±1.14*</td>
<td>35±4*</td>
</tr>
<tr>
<td>120</td>
<td>21±12*</td>
<td>4.57±2.19</td>
<td>31±17*</td>
</tr>
<tr>
<td>Taxol, 50 nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>122±36</td>
<td>5.10±1.44</td>
<td>49±5</td>
</tr>
<tr>
<td>10</td>
<td>118±48</td>
<td>4.25±1.27</td>
<td>51±11</td>
</tr>
<tr>
<td>30</td>
<td>107±105</td>
<td>3.68±0.97</td>
<td>45±13</td>
</tr>
<tr>
<td>60</td>
<td>76±51</td>
<td>4.01±1.13</td>
<td>41±7</td>
</tr>
<tr>
<td>120</td>
<td>153±141</td>
<td>4.35±1.18</td>
<td>45±3</td>
</tr>
<tr>
<td>Control (medium replacement)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>121±48</td>
<td>4.58±1.35</td>
<td>46±8</td>
</tr>
<tr>
<td>10</td>
<td>125±44</td>
<td>4.61±1.24</td>
<td>46±3</td>
</tr>
<tr>
<td>30</td>
<td>156±78</td>
<td>4.75±1.40</td>
<td>45±6</td>
</tr>
</tbody>
</table>

* The values that have a statistically significant difference from the control (independent t-test, \( p < 0.05 \)).

Incubation of cells with nocodazole decreased the quantity of MTs. In 2 h after nocodazole was added, the cells contained only a few MTs diverging from the cell centre. The number of acetylated MTs also decreased. In this case, their quantity in 2 h after nocodazole administration corresponded approximately to the total number of MTs revealed using antibodies to β-tubulin (data not shown).

Incubation of cells with nocodazole increased the number of stress-fibrils in the lamella (data not shown).

Thus, the locomotion of Vero fibroblasts and saltatory movements of intracellular granules depended on the presence of MTs.

Taxol suppresses the locomotion of fibroblasts and disorganizes the saltatory movements of granules. Immediately after the addition of taxol (1 µM) the migration of fibroblasts to the wound was blocked, though they continued to release lamellopodia that advanced by 5 µm on average. Each such advancement of lamellopodia was followed by its retraction.

The number of saltatory movements per unit time decreased gradually.
Figure 4. a. Changes in the standard deviation from the mean of the sample of angles between tracks and the long axis of the lamella before and after the taxol addition (1 (µM); b, distribution of angles between tracks and the long axis of the lamella for different times of cell incubation with taxol (1 µM). The distribution was satisfactorily described by the Gaussian distribution law prior to the taxol addition. In the process of incubation with taxol the Gaussian law became increasingly inadequate for the description of distribution; nonetheless, the distribution was still regarded as Gaussian for the sake of comparison of the parameters selected previously (mean of sample, S.D.).
during the first hour after the taxol addition (Fig. 3b, Table 1). During the second hour of cell incubation with taxol the number of saltatory movement did not virtually change. As the duration of cell incubation in 1 µM taxol increased, the mean length of saltatory movements decreased (Table 1). The ratio of tracks directed towards the nucleus and to the leading edge of the lamella did not change (Table 1).

The most essential change after the addition of 1 µM taxol was the perturbation of predominant orientation of tracks along the lamella long axis (Fig. 3b). The magnitude of standard deviation in the sample increased with the longer time of cell incubation in taxol approaching the standard deviation for the case of equally probable directions (Fig. 4a). The height of the distribution peak dropped dramatically immediately after the addition of taxol and continued to decrease gradually during subsequent incubation (Fig. 4b). In addition, the longer incubation of cells with taxol increased gradually the number of tracks deviating from the longer axis of the lamella to angles close to 90° (Fig. 4b). Hence, after the taxol addition the distribution pattern of the angles of tracks changed, being transformed from the normal to the equally probable.

Thus, in our experiments the movement of fibroblasts was sensitive to taxol and nocodazole. As it was expected, the disassembly of MTs suppresses the saltatory movements and the translocation of cells over substrate. Interestingly, the total stabilization of MTs also represses the locomotion of cells but does not inhibit the saltatory movements and leads solely to their spatial disorganization - a decrease in the frequency of movements along the cell long axis and an increase in the frequency of movements perpendicular to this axis. This may result from both suppression of dynamic instability of MTs and total redistribution of MTs that take place gradually under the action of taxol [17]. It should be noted that under the action of taxol the total number of MTs increases considerably that could also cause disorganization of saltatory movements. For this reason, further analysis of the causes of disorganization of saltatory movements requires the inducement of finer changes in the MT system.

To this end, we used various mitostatics at low (of the order of 100 nM) concentrations. It is known that such concentrations of nocodazole, taxol and vinblastine induce only the suppression of dynamic instability of MTs but do not affect the level of polymerized tubulin [11, 13, 14].

Three different mitostatics were used to reveal what effects follow from the suppression of dynamic instability of MTs and what effects may result from the specific action of each mitostatic.

Changes in the MT system and the active cytoskeleton under the action of low levels of mitostatics. Preliminary experiments have shown that compared to the control no visible changes were observed in the total number of micro-
Figure 5. Variation of the mean rate of the advancement of the leading edge of fibroblasts from the monolayer into the experimental 'wound' after addition of low concentrations of various mitostatics. 1, Control (medium replacement); 2, taxol (50 nM); 3, vinblastine (50 nM); 4, nocodazole (170 nM).

Under normal conditions, the periphery of the lamella of Vero fibroblasts was occupied basically by non-acetylated MTs (Fig. 2b, c). In 10 min after the application of mitostatics at low levels, the acetylated (stable) MTs appeared at the periphery of the lamella (Fig. 2f). This may be indicative of the stabilization of free MTs in the cell.

The quantity of stress-fibrils increased appreciably after the application of nocodazole (170 nM) (Fig. 2d), vinblastine (50 nM) and taxol (50 nM) (data not shown). After 2 h of mitostatic action the number of stress-fibrils became approximately the same as in the norm (Fig. 2g).

**Low levels of mitostatics decrease the rate of fibroblast locomotion**

*Nocodazole (170 nM).* Fibroblasts continued to migrate into the wound throughout the incubation period in the presence of 170 nM nocodazole. However, immediately after the nocodazole addition the mean rate the advancement of the leading edge of cells first decreased considerably and then started to increase, though it did not reach the control value after a 2-h incubation (Fig. 5). The width of the lamellopodia increased upon incubation with nocodazole.
Vinblastine (50 nM). During their incubation with 50 nM vinblastine, the fibroblasts continued to migrate into the experimental wound. During the first 30 min after the vinblastine addition the mean rate of the advancement of the leading edge of cells decreased and then increased virtually to the initial level.
The width of the lamellopodia increased upon incubation with vinblastine.

*Taxol (50 nM)*. Through the entire period of incubation with 50 nM taxol, the fibroblasts migrated into the wound, though at a lower rate than under control conditions. The mean rate of the advancement of the leading edge of cells into the wound diminished to 70% of the control value within the first
30 min after the taxol addition and then remained virtually invariable (Fig. 5). The width of the lamellopodia increased upon incubation with taxol.

**Low levels of mitostatics induce changes in the distribution of the saltatory movements of granules**

*Nocodazole (170 nM)*. In the presence of nocodazole (170 nM) the number of saltatory movements of granules in the lamella decreased during the first hour and then it was stabilized (Table 1). The mean length of tracks and quantitative ratio of tracks directed towards the nucleus and to the leading edge of the cell did not change (Table 1).

Incubation of cells with nocodazole induced the loss of the predominant orientation of tracks along the long axis of the lamella (Fig. 6a). The magnitude of standard deviation of tracks from the mean value increased accordingly, coming close to the standard deviation for the case of equally probable directions (Fig. 7a). The height of the peak of the distribution of the angles between tracks and the long axis of the lamella decreased sharply immediately after the nocodazole addition and continued to diminish gradually.
through the subsequent incubation time (Fig. 8a). In 2 h after the nocodazole application the peak virtually disappeared (Fig. 8a). In addition, in 10 min after the nocodazole introduction the percentage of tracks deviating from the lamella long axis to angles close to 90° increased (Fig. 8a). In the
course of subsequent incubation the quantity of tracks strongly deviating from the long axis remained invariable.

*Vinblastine (50 nM).* In contrast to nocodazole, an appreciable (statistically significant) decrease in the frequency of saltatory movements was observed during the first 30 min after vinblastine was added (Table 1). The mean length of the track decreased after the first hour of incubation and recovered to its initial level by the end of the second hour (Table 1). The share of the tracks
directed towards the leading edge of the lamella decreased immediately after the vinblastine addition and remained virtually constant in the course of subsequent incubation (Table 1).

After the addition of vinblastine, the predominant orientation of tracks along the long axis of the lamella was gradually lost (Fig. 6b). There occurred a gradual increase in the magnitude of standard deviation from the mean value of selection (sample) that was coming close to the standard deviation for the case of equally probable directions (Fig. 7b). The height of the peak of the distribution of the angles between tracks and the lamella axis decreased dramatically immediately after the vinblastine addition (Fig. 8b). The peak nearly disappeared in 30 min after the vinblastine application (Fig. 8b). During the subsequent incubation, the height of the remaining peak was invariable (Fig. 8b). In addition, in 10 min after the vinblastine addition the percentage of tracks deviating from the lamella long axis to angles close to 90° increased (Fig. 8b). The number of strongly deviating tracks did not change in the course of subsequent incubation.

**Taxol (50 nM).** During incubation of cells with taxol the number of saltatory movements of granules decreased slightly (it was statistically insignificant; independent t-test) (Table 1). Throughout the entire period of incubation with taxol the mean length and the ratio of tracks directed towards the nucleus and to the leading edge of the cell did not change (Table 1).

Incubation of cells with taxol led to a gradual loss of the predominant orientation of tracks along the long axis of the lamella (Fig. 6c). The magnitude of standard deviation increased gradually approaching that for the case of equally probable directions (Fig. 7c). The height of the peak of the distribution of the angles between tracks and the lamella long axis decreased sharply immediately after the taxol addition (Fig. 8c). During subsequent incubation the peak height continued to decrease, and in 2 h after the taxol addition the peak virtually disappeared (Fig. 8c). Besides, in 10 min after the taxol addition the share of the tracks deviating from the long axis of the lamella to angles close to 90° increased (Fig. 8c). The percentage of strongly deviating tracks remained invariable in the course of further incubation.

Thus, after the addition of nocodazole, vinblastine or taxol at low concentrations all the directions of saltatory movements of granules in the lamella became gradually equally probable.

**Addition of the mitostatic-free medium does not affect the mobility of cells and the saltatory movements of granules.** Introduction of a mitostatic-free medium did not induce any decrease in the rate of advancement of the leading edge of cells into the wound (Fig. 5). The lamellipodial zone did not increase. Furthermore, the number of saltatory movements of granules and the mean length of their tracks did not change, no did the ratio of tracks directed towards the nucleus and to the leading edge of the cell (Table 1). After the medium replacement the majority of tracks of saltatory movements of
granules remained to be oriented along the long axis of the lamella (Fig. 6d). The standard deviation from the mean value of the sample did not increase, and the distribution peak exhibited no decrease, either (Figs. 7d, 8d).

DISCUSSION

Translocation of fibroblasts. MTs are indispensable for the movement of fibroblasts over substrate. Indeed, upon disassembly of MTs, e.g. under the action of high concentrations of colcemid or nocodazole (in our experiments - 1.7 µM nocodazole), the migration of fibroblasts was stopped. It is interesting to note that not only the disassembly but also stabilization of MTs under the action of taxol (1 µM) induces total inhibition of fibroblast translocation. Consequently, the availability of MTs per se is necessary but not sufficient for the locomotion of fibroblasts.

The common response of Vero cells to low doses of nocodazole, vinblastine and taxol (170, 50 and 50 nM, respectively) was the decrease in the rate of cell translocation during the first 30 min. This is in agreement with the data of Liao et al. [15] who showed that the application of nocodazole (100 nM) and taxol (50 nM) also decreased the rate of locomotion of the NRK line fibroblasts.

The fact that the low levels of mitostatics induced only a decrease in the migration rate but not its total suppression can apparently be explained by the involvement of several mechanisms in the process of fibroblast locomotion. The switch-off of one mechanism leads to a decrease in the migration rate, which is nonetheless maintained by the remaining operating mechanisms.

In contrast to the NRK cells, in the Vero fibroblasts the decrease in the rate of cell migration immediately after the addition of nocodazole and vinblastine was followed by its partial recovery in the process of further incubation with these mitostatics. Apparently, the decrease in the migration rate is the primary effect of these compounds, whereas its recovery results from compensatory changes in the cell in response to the action of mitostatics. After the addition of nocodazole (170 nM) and vinblastine (50 nM) the decrease in the rate of Vero fibroblast migration was followed by its partial recovery, and this once again indicates that several independent mechanisms provide for the locomotion of fibroblasts. The rate of fibroblast migration may recover owing to the activation of the remaining mechanisms of cell locomotion.

Under the action of taxol (50 nM) the locomotion rate of Vero and NRK fibroblasts did not recover. It is possible that this results from specific properties of taxol. Comparison of the graphs of the rate of fibroblast migration into the wound upon application of nocodazole, vinblastine and taxol (Fig. 5) suggests that the cells adapt themselves differently to the action of these compounds.
The decrease in the migration rate immediately after the addition of low concentrations of nocodazole, vinblastine and taxol correlated with the appearance of stress-fibrils in the cell. Irrespective of whether a partial recovery of the rate of cell locomotion took place (nocodazole 170 nM, vinblastine 50 nM) or not (taxol 50 nM), in 2 h after the mitostatic addition the quantity of stress-fibrils became approximately the same as in the control. Apparently, the increase in the quantity of stress-fibrils was the fast response of the cell to the changes induced by the mitostatic. The recovery of the initial quantity of stress-fibrils seems to provide evidence of the completion of cell adaptation. Taking into account that the stress-fibrils are characteristic of immobile or low-mobile cells, it may be suggested that their growth leads to a rapid inhibition of fibroblasts and then, with disassembly of additional stress-fibrils, the rate of their locomotion somewhat increases.

According to Liao et al. [15], nocodazole added at a concentration of 100 nM does not induce any changes in the mass of polymerized tubulin (disassembly of MTs), while the cell locomotion rate decreases to 60% of that in the control. In our experiments with low concentrations of nocodazole, vinblastine and taxol the decrease in the rate of cell translocation was not accompanied by the decrease in the quantity of MTs in the cells, either. This demonstrates once again that the availability of MTs per se is not a sufficient condition for the locomotion of fibroblasts.

It is known that low concentrations of mitostatics used in our experiments suppress the dynamic instability of MTs [11-14]. Thus, the decrease in the rate of cell locomotion soon after the addition of various compounds, which have the only common property - inhibition of the dynamic instability of MTs, indicates that the dynamic instability of MTs is significant for the translocation of cells.

Under the action of low levels of mitostatics, the total quantity of visible MTs did not change; however, the number of acetylated MTs increased (Fig. 2c, /, i). Suppression of the dynamic instability of MTs leads to the longer MT lifetime. This results in a higher probability of chemical modifications of MTs, one of which is acetylation of tubulin [18]. The larger quantity of acetylated MTs suggests the increase in the lifetime of MTs resulting from the suppression of dynamic instability.

Thus, complete disassembly and total stabilization of MTs suppresses the locomotion of fibroblasts, whereas suppression of their dynamic instability only inhibits their movement. It is however unclear what intracellular mechanisms could explain this phenomenon.

It has turned out that considerable modifications of the pattern of saltatory movements of intracellular granules occur simultaneously with changes in the locomotion of cells.

**Saltatory movements of granules.** The tracks of saltatory movements of granules in the Vero fibroblast lamella were arranged in a definite manner:
the overwhelming majority of movements were parallel to the long axis of the lamella, i.e. they were axial (Fig. 1a). In a similar mode, vesicles and mitochondria move in the axon along its long axis in two directions: towards the nucleus and, inversely, towards the axon growth cone [6, 9]. But in a narrow axon, vesicles and mitochondria cannot move to considerable distances across it. The axial character of the tracks of granule movement in the lamella of fibroblasts is not evident. The lamella is sufficiently wide to allow the movement of granules across it.

Spatial organization of saltatory movements of organelles determines the distribution of MTs. In an axon, the MTs are arranged along its long axis. In melanophores, they diverge radially from the cell centre. In the melanophores, pigment granules move radially in two directions: from the nucleus to the cell periphery and, inversely, from the cell periphery to the nucleus [20, 21]. In the lamella of fibroblasts, the MTs are arranged along the long axis of the lamella [22]. Therefore, the tracks of saltatory movements of granules are also arranged along the lamella long axis.

The effects of perturbation of the granule movement along the long axis of the lamella were similar for all the mitostatics used at low concentrations in our experiments. Prior to the mitostatic addition, the angles between tracks and the long axis of the lamella were not equally probable; instead, angles close to 0° were predominant. The mitostatic addition induced an immediate appreciable decrease in the quantity of tracks arranged along the long axis of the lamella and an increase in the number of tracks arranged across the long axis. In the course of subsequent incubation the quantity of tracks arranged along the long axis continued to decrease. As a result, in 2 h after the mitostatic addition the directions of tracks relative to the long axis of the lamella became equally probable.

Thus, similar in their character and dynamics, the perturbations of the movements of granules in the lamella observed soon after the addition of various compounds that have the only common property - suppression of dynamic instability of MTs - makes it possible to conclude that the MTs should possess dynamic instability to allow an ordered (axial) movement of granules.

**Relationship between the dynamic instability of MTs and saltatory movements of granules.** To explain the relation between the dynamic instability of MTs and the axial pattern of granule movement in the lamella we propose the following model.

It is known that the MTs are arranged in the lamella mostly parallel to its long axis ([22], Fig. 2b). The total quantity of MTs per cell is not less than a few hundreds [23, 24]. The frequency of saltatory movements of large granules in intact fibroblasts is 25-30 per minute (Table 1). Thus, if we assume that the movement of granules along MTs occurs randomly, then one granule (visible in the phase contrast) moves along a microtubule approximately
once in 10 min, on the average. Proceeding from this assumption, it may be suggested that one MT has time to 'survive' only one saltatory movement of a large granule. The free MTs are relatively rare in fibroblasts [23, 24]. If we assume the mean MT lifetime to be about 5 min, then the lifetime of free MTs should be even shorter. Hence, it turns out that under normal conditions most of the granules move along MTs fixed on the centrosome. Such tubules fixed on the centrosome are stable. Stable MTs are arranged along the long axis of the lamella [25]. The tracks of granule movements along such MTs will also be arranged along the long axis of the lamella (Fig. 1a).

When the dynamic instability is suppressed, the lifetime of free MTs obviously increases. The longer lifetime of MTs increases the probability of their chemical modification, e.g., acetylation [18]. Indeed, in the course of incubation in the presence of mitostatic, the quantity of acetylated MTs increased (Fig. 2c, f, i). As a result of the longer lifetime of free MTs, their quantity per cell increased considerably. Then the movement of granules may occur to a considerable extent along the free MTs.

Let us consider the transport of a granule along a free MT. The mass of a mean granule 0.8 µm in diameter is:

\[ m_g = \rho_g V = \frac{4}{3} \pi \rho_g r^3 \approx 0.2679 \rho_g \mu m^3, \]

where \( m_g \) is the granule mass; \( \rho_g \) is the granule density; \( V \) is the granule volume; \( r \) is the granule radius. The mass of a mean MT 10 µm in diameter is:

\[ m_m = \rho_m V = \pi \rho_m r^2 h \approx 0.0049 \rho_m \mu m^3, \]

where \( m_m \) is the MT density; \( \rho_m \) is the density of MT; \( V \) is the MT volume; \( r \) is the radius of MT; \( h \) is the MT length. The densities of the granule and MT are approximately equal.

According to the law of pulse preservation upon movement of a granule along a non-fixed MT, the latter will dislocate in the direction opposite to the granule movement. As a result of such dislocation, the MT may collide with some cell organelles, the density of which in the cell may be sufficiently high. Since the force developed even by a single motor molecule is rather high (0.12 pN by kinesin [26]), the consequence of such collision may be a turning of MT or its bending. Hence, movement of a large-mass granule may result in the dislocation of a free MT and change in its position or lead to its bending. This may be conducive to a gradual disorganization of the distribution of tracks that was in fact recorded by us.

We suppose that there is a relation between the suppression of dynamic instability of MTs common for low doses of mitostatics and the loss of
predominant distribution of tracks along the long axis of the lamella.

In the first approximation, it may be stated that we observed a two-phase response. The share of the tracks deviating from the long axis of the lamella increased rapidly immediately after the mitostatic addition, while after 30 min it reached a plateau or continued to increase insignificantly. After 90-120 min the distribution of tracks in the lamella became totally chaotic. If we assume the mean lifetime of an individual MT in a normal Vero cell to be about 5 min or less, it is obvious that the suppression of dynamic instability will immediately lead to an appreciable extension of the lifetime of free MTs. Thus, after 10-30 min the cell will accumulate a considerable quantity of free MTs that will gradually be chaotically redistributed as a result of their interaction with the motors.

**Relationship between the saltatory movements of granules and the locomotion of fibroblasts.** A hypothetical explanation of the MT role in the translocation of fibroblasts may be organization of the transport of membrane vesicles from the Golgi apparatus to the leading edge of the cell. Such a transfer can lead to the incorporation of vesicles into the cell membrane at the cell leading edge and result in the increase in the membrane area and growth of lamellipodia in a strictly definite direction. It was reported earlier [8] that the repression of transport towards the leading edge of the cell during the kinesin inhibition leads to the suppression of the lamellopodial activity of the leading edge of the cell.

Perturbation of the saltatory movements of the granules studied in our experiments seems to reflect disorganization of the entire transport along the microtubules. At the same time, disorganization of the transport of organelles at the invariable frequency of movements means a less effective transport towards the leading edge of the cell that ensures the cell translocation.

Thus, our findings indicate that the dynamic instability of MTs in polarized fibroblasts is necessary for the maintenance of the axial distribution of the tracks of saltatory movements of granules and, by mediation through transport over MTs, plays an important role in the dislocation of fibroblasts over substrate.

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