AENSI Journals

Australian Journal of Basic and Applied Sciences

ISSN:1991-8178

Journal home page: www.ajbasweb.com

Hypothesis about : cleavage furrow- pressure differences determine cleavage furrow position

[1]Abdallah Radwan ben-Aabdallah , [2]Pierre Schembri-Wismayer, [3]D.S.sheriff

[1]MBBS student, Faculty of Medicine, Benghazi University, Benghazi, Libya .
[2].Anatomy |Dept, Faculty of Medicine & Surgery, University of Malta.
[3]Department of Biochemistry, Faculty of Medicine, Benghazi University.

ARTICLE INFO

Article history:
Received 2 March 2014
Received in revised form
13 May 2014
Accepted 28 May 2014
Available online 23 June 2014

Keywords:
Cleavage furrow , Pressure difference, unstable region, chromatids ,Actinomyosin, anaphase, Proteins GEFs, CDK-1.

ABSTRACT

Cleavage furrow forming and position in animal cells is highly dependent on the position of mitotic spindle as is shown by many studies. There is evidence that external pressure has an important role in defining the position of mitotic spindle which determines the axis of division. It is being hypothesised that the extracellular pressure which compresses the divided cell membrane has a pivotal role in formation of cleavage furrow and determining its position. It is likely this due to the pressure difference between the inside and outside the cell created during anaphase as a result of precise highly ordered cellular processes. As a result of this difference, the resulting force will compress the middle of the cell membrane from the outside, causing polarization of actin units and cytokinesis.

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To Cite This Article: Abdallah ben-Aabdallah, Pierre Schembri-Wismayer, D.S.sheriff., Hypothesis about : cleavage furrow- pressure differences determine cleavage furrow position. ***Aust. J. Basic & Appl. Sci.,*** *8(10): 628-637, 2014*

INTRODUCTION

The cell is the basic unit of the body and the functions of the assembled cells determine the various tissue functions of the human body. Thus our lack of understanding of certain cellular actions especially during cell division could hamper our understanding of many diseases, such as cancer.

The process of cell cleavage is an accurate process relevant to cancer which is a disease of uncontrolled cellular proliferation. A better understanding of the formation of the of cleavage furrow will enhance our knowledge of cancer and how this excess proliferation can be targeted.

The present small write up discusses the effect of external force in defining the position of the cleavage furrow and the ability of the cell to control the formation of this force during anaphase.

Hypothesis:

The chromatids' separation during anaphase causes a mechanical tension or cytosol displacement, forming a low pressure area (unstable region) where the chromosomes had lined up before separation. Microtubules slow inward cytosolic flow to fill this region upon chromosome migration to the poles during anaphase, which leads to the difference in pressure between inside and outside the cell, causing an invisible pressure belt pressing on the cell membrane from the outside. In response to this pressure, actin begins to accumulate. It was also shown that external cellular constraints induce a polarization of dynamic subcortical actin structures that correlate with spindle movements (Fink J, Carpi N, Betz T, Bétard A, Chebah M, Azioune A, Bornens M, Sykes C, Fetler L, Cuvelier D, Piel M, 2011).

Forces exerted on mitotic Dictyostelium discoideum cells in another study induced recruitment of the myosin II and actin cross linker cortexillin I, followed by local contraction (Effler JC, Kee Y-S, Berk JM, Tran MN, Iglesias PA, Robinson DN, 2006), resulting in a ring of actin units and thereby determining the position of the cleavage furrow.

The continued presence of the unstable region in the late stages of cytokinesis helps actin contract and also works to reduce the vector impact pressure from the inside of the cell to its outside due to actin-induced tightening of cell membrane thereby causing formation of negative pressure in the surrounding medium of the cell like increase negative pressure in the pleural cavity during inhalation.

Corresponding Author: Abdallah Radwan ben-Aabdallah, MBBS student, Faculty of Medicine, Benghazi University, Address: Benghazi, Libya
E-mail: abdallahradwan777@yahoo.com

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Formation of Unstable Region:

The seperation of the chromosomes during the anaphase makes a disturbance & movement in the cytosol in the area existing in ,thats via the the waves produced by this chromosomes pushing one & another to the poles,not to underestimate the numbers of the chromosomes 46 in each pole adding to this it stong structure due to the DNA density ,and by looking to the site of the centriole in the periphery & the chromosomes arranged in the center ,thus while the centriole is dragging the chromosomes they will compres each other leading to formation of a mechanically effective mass & will drag an amount of the cytosol with it,making disturbance with high effect.

The polar microtubules pass inbetween the arranged chromosomes in the metaphase,and when they start to seperate,they start to compres each others ,thus compressing the microtubules between them , and that making bundles of microtubules ,wich will be a place to creat an unstable region & maintain it due to the geometrical structure of this bundles.

This disturbance or the unstable region occured due to the movement of the chromosomes surounding these bundles adherently ,& while dragging the chromosomes attached to the polar-microtubules it will evacuate an amount of the cytosol inbetween the polar-microtubules,thus due to the disturbance that will be made by the movement of the surrounding chromosomes to the bundles.

after exciting an amount of the cytosol inbetween the microtubules forming the bundles,the disturbance surrounding them will stop,& the cytosol will start to compress the bundles due to the unstable region inbetween the microtubules after the evacuation ,but because of the shortness of the distance between the microtubules the cytosol will not enter & the unstable region will be preserved for quite a while.

There are some reasons that will let the cytosol goes out from the polar microtubules and at the same time will not allow cytosol to return again..

1-What made the evacuation process or let the cytosol comes out is the force of the disturbances outside ,which made a pressure differences between outside & inside the bundles ,thus this diffrence will bush from the inside more ,creating more space inbetween the microtubules and so cytosol will move outside of the bundles easily and so causing formation of unstable region .

2-Regarding the maintance of the cytosol from entering inbetween the microtubules after formation of the unstable region is due to to the force thats pushing to the inside thus reducing the space between the microtubules & prevent the cytosol from entering

N.B some of the cytosol may enter ,but its very diluted & resembles water & lacks some components

Discussion:

With development of modern measurement techniques, scientists could identify the pressure or the vector force acting on the cell and generated by the surrounding environment (interstitial fluid). Much research has shown a role of the force over the cell to attract and activate many proteins and enzymes which play an important and pivotal role in forming a cleavage furrow, amongst these elements: actin and myosin as we will discuss later.

Studies have proven that external force controls the mitotic spindle position which plays a role in determining the axis of the division (Fink J, Carpi N, Betz T, Bétard A, Chebah M, Azioune A, Bornens M, Sykes C, Fetler L, Cuvelier D, Piel M , 2011).This clearly indicates that cells need pressure during division. Animal cells decide where to build the cytokinetic apparatus by sensing the position of the mitotic spindle (von Dassow G., Verbrugghe K. J., Miller A. L., Sider J. R., Bement W. M , 2009).

When the cell starts dividing, there are many biochemical and morphological changes that occur which play an important role throughout mitosis to ensure a cleavage furrow correctly starting and eventually ending through the assembly and constriction of actin.

At the end of anaphase we note gathering of actin units at mid-cell to form a contractile ring. The concentration of actin fluorescence in the equatorial region, accompanied by a decrease of fluorescence in polar regions, is detected 2-3 min after the onset of anaphase (Cao, L.-G., and Y.-L. Wang, 1990). Amongst the possible causes of actin mobilization we find biochemical signals such as those from Rho GTPases. Rho GTPases are molecular switches that control a wide variety of signal transduction pathways in all eukaryotic cells. They are known principally for their pivotal role in regulating the actin cytoskeleton and their ability to influence cell polarity (Etienne-Manneville S, Hall A, 2002). Rho is a critical regulator of cleavage furrow induction, and its activity is required for cellular cortical furrowing (Mabuchi, I., Hamaguchi, Y., Fujimoto, H., Morii, N., Mishima, M., and Narumiya, S , 1993). We already indicated that physical forces also play a role in subcortical actin polarization (Fink J, Carpi N, Betz T, Bétard A, Chebah M, Azioune A, Bornens M, Sykes C, Fetler L, Cuvelier D, Piel M , 2011).

Active Rho accumulates in a narrow cortical band overlying the mitotic spindle, and spindle displacement leads to repositioning of this cortical band of active Rho (Bement, W.M., Benink, H.A., and von Dassow, G, 2005).

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Other studies show that application of force on integrin proteins stimulates the RhoA pathway through a number of guanine nucleotide exchange factors (GEFs) regulators (Christophe Guilluy, Vinay Swaminathan, Rafael Garcia-Mata, E. Timothy O'Brien, Richard Superfine & Keith Burridge , 2011). GEFs that belong to the Dbl family are key proteins in the activation of the small GTPases of Rho family. This activation allows turning on different signaling pathways such as the differentiation and cellular growth and the cytoskeleton rearrangements (Vargas, M.; González-de la Rosa, C. H , 2007).

Active Rho accumulates in a narrow cortical band overlying the mitotic spindle (Mabuchi, I., Hamaguchi, Y., Fujimoto, H., Morii, N., Mishima, M., and Narumiya, S, 1993) and this is one of the points that refer to presence of an external force kept on the middle of the cell during anaphase.

Increase the size of the cell at the poles facilitates formation of the cleavage furrow by concentrating the pressure across the shortest cellular thickness from the unstable region (in middle) and so determining the cleavage furrow position , and the gathering of actin units in the middle of the cell.

One of the actions which most clearly indicate the ability of the cell to develop an external pressure force pressing on the cell membrane is the separation of chromatids and its movement to the poles of the cell. This causes controlled ripples in the cytosol and the drawing away of small amounts of cytosol from the mid-cell, causing the unstable region between the spindle microtubules, which in turn prevent the return of the displaced cytosol to the area of instability.

In relation to this, microtubule depolymerization during metaphase or very early anaphase prevents cleavage furrow formation (Hamaguchi, Y , 1975).

Unstable region (UR):

This results from the movement of chromatids during anaphase, causing areas of low pressure between microtubules.

Polar microtubules prevent cytoplasmic fluid filling these low pressure pockets (areas where its cytoplasmic content is less dense than other cytoplasm i.e. area which lack many of the components of cellular fluid or areas with reduced cytosol). Together, these low pressure pockets form the unstable region.

Formation of these low pressure pockets together which causes the pressure difference between the inside and outside of the cell, causing the force pushing on the middle of the cell(perpendicular to the mitotic spindle) from the outside on the same stretch of the unstable region, constituting the invisible pressure belt which affects the mid-zone cell membrane from the outside, leading to actin unit accumulation as a response to this pressure, which causes a series of chemical signals, where actin units become distributed in the middle of cell and begin constriction (with help from the trans membrane pressure difference).

Formation of unstable region is like using small injection to suck olive oil, the olive oil will not pass to the container easily, although that there is pressure difference but this pressure is not adequate to push the olive oil to the container because of density of the oil and the diameter of the opening of the needle is very narrow, here microtubules work exactly like the needle.

Studies have found that extracellular pressure causes activation of Rho-GEF proteins, which in turn play an important role in determining the focus of the division and the movement of subcortical actin structures. It was shown that the stimulation of integrins with tension force triggers the activation of these two GEFs (LARG and GEF-H1) and their recruitment to adhesion complexes (Christophe Guilluy, Vinay Swaminathan, Rafael Garcia-Mata, E. Timothy O'Brien, Richard Superfine & Keith Burridge, 2011). GEF activation turns cytoskeleton rearrangements (Vargas, M.; González-de la Rosa, C. H 2007), the return of cytosol to the unstable region recreates the stability of the cell i.e. pressure difference disappears.

The role of the Microtubules may be likened to the underwater spider web of Argyroneta aquatica nest which holds air bubbles –without allowing bubbles to escape between spider webs, so the microtubules prevent the displaced cytosol from returning to unstable region exactly like web prevents bubbles to arise to the surface.

Cellular constriction would be very difficult for Actomyosin units in the absence of this unstable region. Should this unstable region not form during the process of Anaphase and had actomyosin units accumulated in the mid-cell during the process of Anaphase (by whatever means), they would have to pull the membrane inwards without a pressure difference, this would result in formation of negative pressure in region around the outer mid-cell and as a result of this, the pressure difference generated between inside and outside the cell would be reversed with increased pressure inside, making it difficult for actin units to constrict and difficult to complete cytokinesis. In fact, in Caenorhabditis elegans embryos, disruption of the central spindle does not prevent cleavage furrow ingression. Under these conditions cleavage furrows form and constrict, but they fail to complete cytokinesis (Powers, J., O. Bossinger, D. Rose, S. Strome, and W. Saxton, 1998) (Raich, W.B., A.N. Moran, J.H. Rothman, and J. Hardin, 1998) (Jantsch-Plunger, V., P. Gönczy, A. Romano, H. Schnabel, D. Hamill, R. Schnabel, A.A. Hyman, and M. Glotzer, 2000), showing the importance of microtubules in generating a low pressure zone in the cell Actomyosin contraction. In addition to this, the amount of interstitial fluid plays an important role in forming the cleavage furrow and determining its shape.

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A recent study found that external force causes a rotated mitotic spindle during some stage of division before anaphase resulting in determination of axis division (Fink J, Carpi N, Betz T, Bétard A, Chebah M, Azioune A, Bornens M, Sykes C, Fetler L, Cuvelier D, Piel M, 2011), This evidence suggests that the cell needs the presence of an amount of pressure perpendicular to the mitotic spindle, suggesting that the interaction between the cell and its environment, plays an important role in attracting actin units and maybe influencing cell fate.

However, in many specialized cell types or under certain environmental conditions, the cell division machinery is placed at non-medial positions to produce daughter cells of different sizes and in many cases of different fates (Snezhana Oliferenko, Ting Gang Chew, and Mohan K. Balasubramanian, 2009). This may result in cell division being symmetric or asymmetric.

The cell division axis therefore determines the future size and positions of daughter cells and is thus critical for cell fate (Thery M, Racine V, Pepin A, *et al*, 2005).

The movement of chromatids to the poles of the cell forms wake- like shapes from the chromosomes. The higher the number of chromosomes, the better they are in encouraging cleavage furrow formation and possibly therefore enhancing cell division.

We will hypothesize that a relationship exists between the efficacy of cell division and a number of cellular features including cell dimension, the size, number and structure of chromosomes and cytosol viscosity. One speculates that a graph drawn between cytosol viscosity as x-axis and cells successfully completing cytokinesis on the y-axis (ignoring cell cycle) would show a rise from low viscosity to peak in the middle and then drop down again at high viscosity because at low viscosity, chromatids do not pull cytosol easily to the poles because of its liquidity, (and also easy flow of the cytosol into any low pressure pockets formed) this would hamper the formation of the unstable region (central low pressure) resulting in no pressure difference leading to failure in occurring of cytokinesis for most cells. At a higher viscosity the efficiency of chromatids to withdraw cytosol towards the poles at anaphase is increased, causing the unstable region which at that viscosity will also not easily fill, thus keeping the unstable region as long as possible and creating the pressure difference. At very high viscosity, chromatid separation during anaphase, is hindered so it should reduce cell division efficacy.

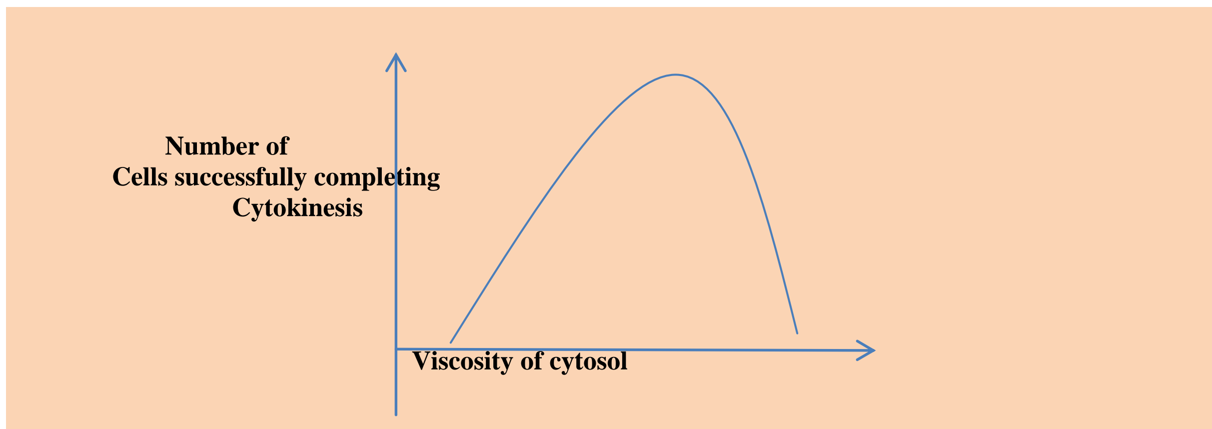

Fig. 1: Indirectly shows the effect of unstable region on cytokinesis.

There is another aspect which may be considered in this hypothetical , is the change in cleavage furrow shape with increasing viscosity.

The reason behind ignoring the time factor (the cell cycle) is action of enzymes inside the cell, one of the techniques that used to increase the viscosity is decreasing the temperature and that will affect in the action of enzyme and make the time of division longer. Even if there are other techniques to increase the viscosity without causing decrease in temperature we have to ignore the time, because the viscosity itself will decrease the motility of important biomolecules.

Among the factors that can increase the viscosity of the cytoplasm is the nuclear envelope disintegration , which will cause the nucleoplasm (considered to be of a high viscosity) to merge with the cytoplasm, and this factor will be very effective in increasing cytosol viscosity, especially in cells with a central nucleus and symmetrical division.

When cells exist in a confined space such as ovum inside the zona pellucida, cleavage furrow formation takes another form, due to the same principles discussed above. As the developing ovum enters Anaphase 1, the accumulated homologous chromosomes begin separation from each other and try to form the unstable region with assembling of actin units. However, for chromosomes to be pulled apart, at the center, the force would

need a large unstable region to be neutralized by the influx of cell membrane under pressure from the extracellular fluid. However due to the lack of extracellular fluid, the pushing in is limited and thus one can imagine the constriction ring sliding to the very edge of the cell to reduce negative pressure in the surrounding medium and so the limited pressure would cause cytokinesis to form the polar body near the cell membrane.

Conclusions:

Cleavage furrow formation depends not only on the existence of an internal unstable region but is heavily dependent on enzymes and biomolecules responsible for organizing the division. In some cases cleavage furrow formation could happen in the absence of this unstable region.In such cases the cell would be more susceptible to failure in cytokinesis and would require some other feature such as another mechanism of balancing intracellular and extracellular pressures, such as the pumping of large amount of intracellular solutes into the extracellular fluid, thus reducing intracellular pressure whilst increasing extracellular fluid pressure. In the absence of this kind of situation, the formation of an unstable region of low pressure in the centre of the cell between the retreating chromosomes being pulled to the poles causes enough of a pressure difference to aid the invagination of the cell membrane.

The need for formation of unstable region may mainly be based on the size of the cell, the amount and the distribution of extracellular matrix that surrounds cell and the shape of division (Symmetrical or asymmetrical division).

Determination The Time Of Division:

Without an unstable region there will not be external pressure belt. But that does not mean there is not enough pressure, of course there is pressure (pressure that result from natural distribution of extracellular fluid and causes mitotic spindle rotation before the separation of chromosomes) but this pressure is not enough surrounding the cell to complete a strong belt. So one of the functions of unstable region is to magnify and regulate the shape of this pressure and so the shape of contractile ring.

As shown above that cells responds to pressure which causes attraction of actin units and some of other modulator like guanine nucleotide exchange factor H1(GEF-H)1.

This raises a very important question.

Although that there is pressure exerted on the cell even during metaphase, What is the thing that make cells form cleavage furrow after formation of unstable region?!!!

The first answer that will come to our minds is the pressure exerted by the presence of unstable region is strong enough to activate actin units and forming cleavage furrow , but the most likely answer is presence of a timer like cyclin dependent kinase (CDK1) is one of the keys that work to determine the response time of pressure

The mitotic kinases Aurora A/B and Cdk1/Cyclin B phosphorylate GEF-H1, thereby inhibiting GEF-H1 catalytic activity (Jörg Birkenfeld, Perihan Nalbant, Benjamin P. Bohl, Olivier Pertz,,Klaus M. Hahn, and Gary M. Bokoch , 2007). Normally CDK1 activity decreases in anaphase, and so allow GEF-H1 to work.

Our results identify a GEF-H1-dependent mechanism to modulate localized RhoA activation during cytokinesis under the control of mitotic kinases (Jörg Birkenfeld, Perihan Nalbant, Benjamin P. Bohl, Olivier Pertz,,Klaus M. Hahn, and Gary M. Bokoch , 2007).

GEF-H1 was required for RhoA activation and intracellular actin/myosin contractility in response to increased matrix stiffness (Heck JN, Ponik SM, Garcia-Mendoza MG, Pehlke CA, Inman DR, Eliceiri KW, Keely PJ , 2012).

And that means inhibition of CDK1 will allow GEF-H1 to work and so will cause of formation of cleavage furrow even before chromosomes separation (without presence of unstable region). In this case GEF-H1 works because of pressure that result from natural distribution of extracellular fluid before the separation of chromosomes (what we mean is whatever the source of pressure, the pressure itself determines cleavage furrow position and this determination happen successfully by presence of unstable region which make it more organized) and that is what shown by the study ,where inhibition of CDK1 by some of inhibitors like BMI-1026 will cause formation of cleavage furrow even before separation of chromosomes. Furrow ingression was initiated before chromosome separation in BMI-1026-treated cells (Niiya F, Xie X, Lee KS, Inoue H, Miki T, 2005).

As we know there is enough amount of pressure surrounds the cell, it is the same pressure that causes rotation of mitotic spindle, and it will be the same that cause stimulation GEF-H1 and forming cleavage furrow which is irregular in the shape and the position because of irregular disruption of extracellular matrix.

BMI-1026-treated cells did not undergo abscission. A population of BMI-1026-treated cells did not undergo abscission, and the emerging daughter cells subsequently merged to generate cells with aberrant nuclei (Niiya F, Xie X, Lee KS, Inoue H, Miki T , 2005) and causing formation of ectopic cleavage furrows . However, in the presence of a low concentration of nocodazole, BMI-1026 induced excessive membrane bleb, which appeared to

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be caused by formation of ectopic cleavage furrows (Niiya F, Xie X, Lee KS, Inoue H, Miki T, 2005). So CDK1 acts like the key to control the time of GEF-H1 activation.

CDK1 Inhibition precociously induces cleavage furrow ingression in Mitotic Cells (Niiya F, Xie X, Lee KS, Inoue H, Miki T, 2005).in this case GEF-H1 may cause initiation cleavage furrow formation. But in normal cases may be GEF-H1 is not responsible for initiating cleavage furrow ,because the activation of GEF-H1 occurs during the onset of anaphase.

During the metaphase to anaphase transition, anaphase-promoting complex/cyclosome inactivates cyclin-dependent kinase (CDK)1 through cyclin B degradation. In anaphase, sister chromatids separate and move to opposite spindle poles as CDK1 activity decreases (Niiya F, Xie X, Lee KS, Inoue H, Miki T, 2005). And that does not mean GEF-H1 does not have a role in cytokinesis, because perturbation of GEF-H1 causes impaired cytokinesis. Perturbation of GEF-H1 function induces mitotic aberrations, including asymmetric furrowing, membrane blebs, and impaired cytokinesis (Jörg Birkenfeld, Perihan Nalbant, Benjamin P. Bohl, Olivier Pertz,,Klaus M. Hahn, and Gary M. Bokoch, 2007).

So until we find the molecule that initiates this whole process, which in normal cases respond to surrounding pressure, it is better to say unstable region attracts actin units.

All of this, shows that the cells have not only one mechanism to respond to surrounding pressure to determine or formation of cleavage furrow. In other words there are more than one biochemical or biophysical processes that are involved in cytokinesis and these processes are interconnected with each other. i.e. ablation of one will affect any of the processes - cleavage furrow formation or cytokinesis and abscission.

Coordination between the effective pressure that coincides with separation of chromosomes and the biomolecules especially that are found in mitotic spindle may determine two important phenomena: the site of cleavage furrow formation and the time , and both together work to make the division successful.

Over All Hypothesis Requires:

1- Regulated effective pressure is created by unstable region.

2- One or more elements respond to pressure and initiate to convert this mechanical energy to chemical energy by a cascade of activation ending by activating (Rho) proteins leading to actin accumulation and contraction.

3- The timer : may be a protein which regulate the time of response.

The presence of an unstable region with a defect in the mechanism that converts mechanical energy to chemical,will inhibit cleavage furrow formation. And the other hand, the present of such pressure transducers without the unstable region would lead to ectopic cleavage furrows, because this substance will respond to surrounding pressure without localization.

*****Linking between the ability of the cell to divide with laws of physics is very intelligent**:***

We think that this hypothesis explains polar relaxation hypothesis and make it more clear and may give an answer about how microtubules can simultaneously function as both positive inducers and negative regulators of cytokinesis (Microtubules stimulate contractile-ring formation in the equatorial cortex and simultaneously suppress contractility in the polar cortex), and that because GEF-H1 is a microtubule-regulated exchange factor that couples microtubule dynamics to RhoA activation (Jörg Birkenfeld, Perihan Nalbant, Benjamin P. Bohl, Olivier Pertz, Klaus M. Hahn, and Gary M. Bokoch, 2007). GEF-H1 is localized to the mitotic apparatus in HeLa cells, particularly at the tips of cortical microtubules and the midbody (Jörg Birkenfeld, Perihan Nalbant, Benjamin P. Bohl, Olivier Pertz,,Klaus M. Hahn, and Gary M. Bokoch, 2007). And as cited above that GEF-H1 response to pressure and its role in rho activation - may play a role in regulating contractile ring shape during late stages of cell division.

Hence a mathematical equation could be envisaged describing the possibility of success of a cleavage furrow from inferred numbers such as the cell diameter, the amount of force which cause actin units to constrict, and pressure difference between the inside and outside of the cell.

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Figure(1&2):

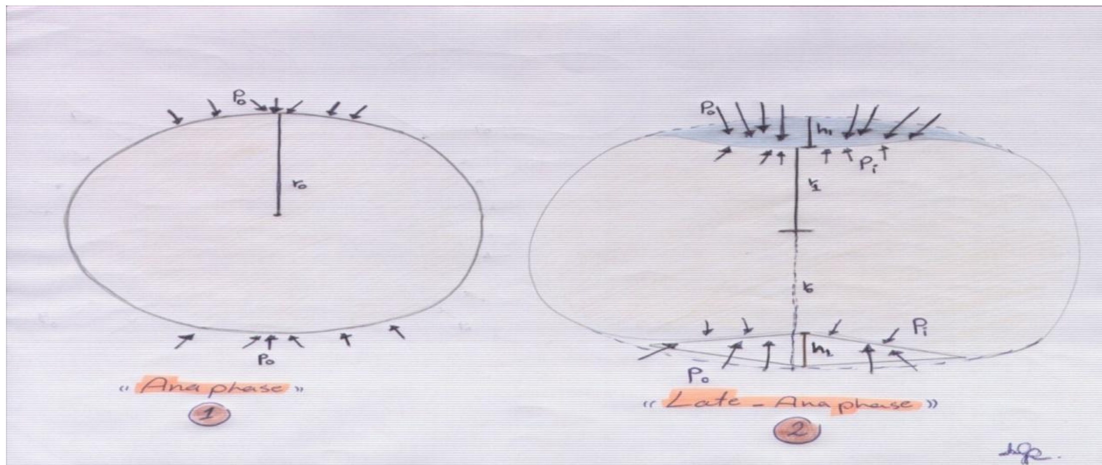

Fig. 1: Shows the beginning of the unstable region in the cell during anaphase where external pressure Po is larger than internal pressure Pi and so pressure difference began pressing on the cell from the outside to inside and this pressure is almost equal to the cell perimeter from outside at first, but after pushing microtubules polar to cell polar leading to collect and focus the pressure at the center as seen in figure 2, because of its proximity to the area of unstable region, thus leading to attract actin units and stimulate a series of interactions that regulate the function of cleavage furrow properly.

Fig. 2: shows that external pressure had been focused on the middle and actin units had started to constrict leading to tighten cell membrane, this tension causes the cell radius decrease from r0 to r1 .This decrease in radius caused increasing the negative pressure in surrounding environment, and as a result of that external pressure will decrease but it still higher than internal pressure Pi that pushs from inside the cell to the outside and because the amount Pi will be smaller than Po at this stage so the pressure difference would be bound from outside the cell to the inside.

The amount of Po decrease with increasing (h) which represents the difference between the initial radius r0 and the radius of the current stage i.e. h1 at this stage equal to (r0- r1).

Figure(3):

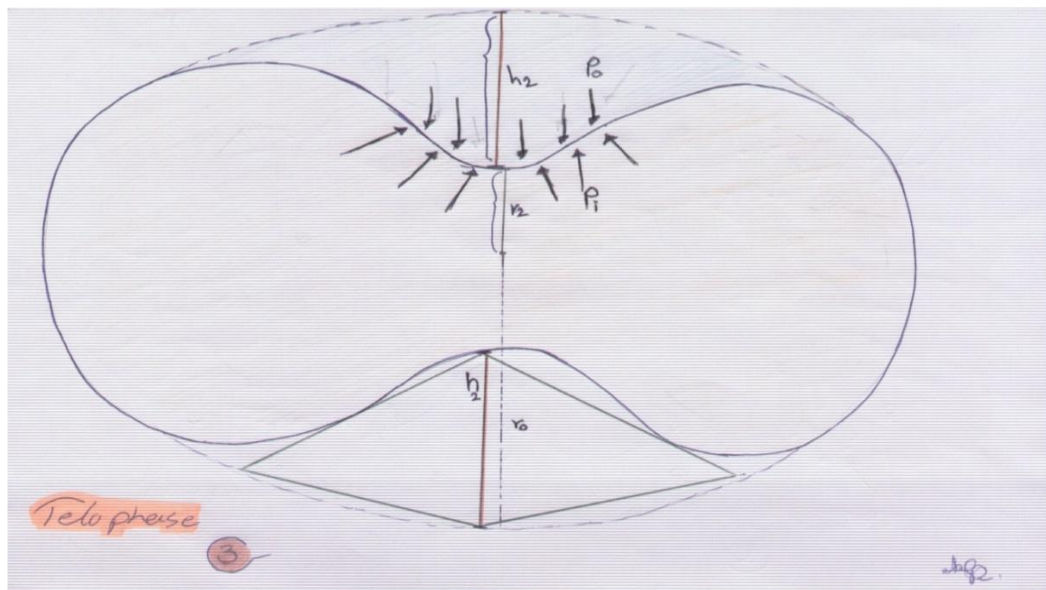

Fig. 3: shows the cell in Telophase where it shows decrease in Po because of increasing tension for cell membrane and so (h) increase and becomes Pi near to Po amount or equal to it, since h2 in this stage equal (r0- r2).

Figure(4):

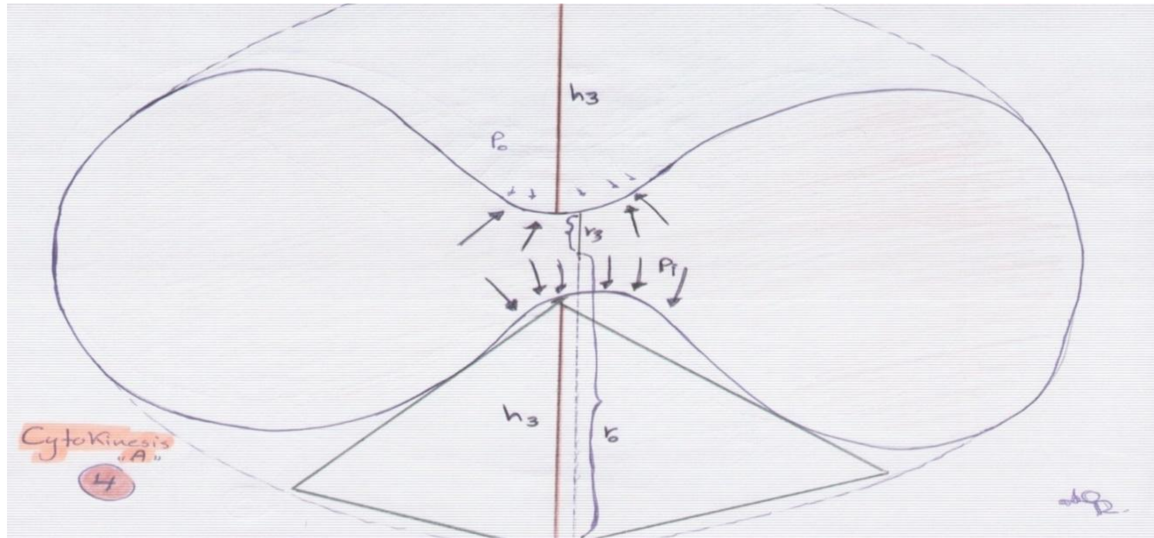

Fig. 4: shows the early stages of cytokinesis, and that the Po amount became smaller than Pi amount and thus pressure difference will be bound from the cell inside to the outside which leads to harder process of the actin units where increasing tensile strength actin units until it reaches the late stages of cytokinesis.

Figure(5):

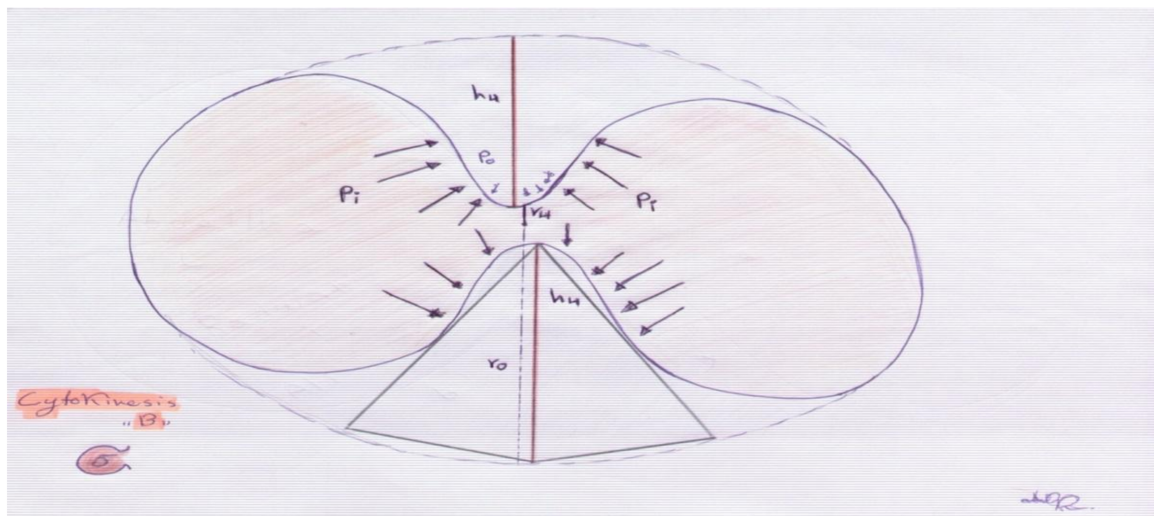

Fig. 5: shows the late stages of cytokinesis, where at this stage pressure amount will be reduced on vector actin units from inside the cell to the outside, Where that if we look at the cleavage furrow we will find that it took the form of a (V) letter from both sides and this form is known geometrically that facing much less pressure than the flat or linear shape.

The absence of unstable region make it difficult for actin units to contract, existence of unstable region helps actin units to reach to late stages of division (delivers it to a stage where a cleavage furrow is not linear).

And to arrange events chronologically, before the cell enters the process of division and after passing prophase and metaphase and entry into anaphase the chromatids begin to move towards the respective poles causing an unstable region in the center of the cell, which results in external pressure compressing the cell membrane (as a result of the pressure difference between inside and outside the cell).

Po: external pressure (pressing on the cell from the outside).
Pi: internal pressure (its direction from inside the cell to the outside).
r0: Initial radius of the cell (before formation of cleavage furrow).
h: Shows decreasing radius of the cell during cytokinesis (r0 - rx) ,where X = (1.2.3.4) .

Thus, unstable region plays many roles, including:

1 - helps in determining the position of the division, depending on the alignment of chromosomes in place during metaphase, (which is responsible for the formation of this unstable region where 46 chromosomes is not a small number in a cell measured by Micron, so it must play a physical role in addition to its role in transfer of genetic material).

2 - stimulation of actin units to assemble, as a result of pressure difference vector from outside to inside the cell on the same stretch of unstable region, forming an invisible pressure belt exerting on the cell membrane of the divided cell from outside; and addition to that ,this pressure could stimulate some key enzymes during Cleavage furrow formation.

3 - during formation of unstable region the outer pressure (po) projecting to the mid-cell bigger in amount than pressure inside the divided cell (pi) (po>pi) when cleavage furrow start forming, the difference starts decreasing gradually until the pressure equalized inside and outside the cell (op=pi). Because actin units pulling of the cell membrane, causing formation of negative pressure in the outer medium.

4 - Unstable region of the divided cell and the semipermeable cells membranes that surrounding the divided cells may enable the Actomyosin unit's contraction. (Helps in the successive occurrence of Cytokinesis) because the surrounding cells excreted amount of substance to intercellular fluid which cause reducing the effect of negative pressure and help actin units to contract.

At the late stages of forming the cleavage furrow the pressure inside the cell becomes large enough than the pressure outside the cell (Po <Pi) helping the cells separation from each other, but that pressure will not prevent the contraction of Actomyosin units because at this stage cleavage furrow formation nearing completion. When we looked to the cell vertically we may find that Cleavage furrow takes the shape of V from both sides, and this figure is known to be exposed to less amount of pressure compared whether it has linear form.

Without unstable region the external pressure is smaller than internal pressure of the divided cell (before that cleavage furrow taking V shape) and so the pressure difference will push from inside to outside and prevent the constriction of actin units.

Method (part of the hypothesis):

In order to infer to the existence of the unstable area we must highlight the pressure indicated by the hypothesis, i.e. the systematic pressure resulting from the presence of the unstable area, which begins its formation during anaphase developed after chromosomes separation which can reach its highest level at the moment of the chromosomes arrival to the both poles of the cell.

In fact the existence of this pressure can be inferred by observing the activity of certain receptors scattered on the surface of the cell that responds to pressure, but due to the lack of our understanding and non-completion of many series of interactions which occur within the cell, especially during splitting, these receptors activity would not provide enough evidence for the existence of this pressure because the activity of these receptors may be affected by other effector within in an indirect way and so making manufactured receptors may be highly sensitive responding only to pressure changes is a good solution .

The experiment (part of the hypothesis):

These receptors must be designed with high accuracy where they should respond only to pressure created on it from one side, and also could be distributed on the external surface of enveloping the cell, or distribution of receptors in localized and confined to a particular part of the cell surface. The most important aspect is that these receptors configure a perfect picture for the pressure distribution and its strength on the membrane during cell division.

The experiment relies on gradual change of existing cytosol viscosity to the pressure changes recorded by receptors during cell division.

Levels of pressure will be recorded on a group of cells during division under normal circumstances, without any change in viscosity in order to have an essential measure for change. Then we'll alter the viscosity by a small amount and gradually for a group of divided cells and look at pressure levels and their distribution where each degree of change in viscosity in a group of cells which are tested under the effect of this change.

In this experiment, the x-axis will represent the amount of cytosol viscosity change and the y-axis represents amount of pressure directed on the middle of the cell for instant access to the two poles of the cell chromosomes (an event which refers indirectly to complete formation of the unstable area).

The timing of the cells to divide will be ignored, due to the impact of low temperature that will affect the activity of enzymes. Reducing the temperature can be used as a means to increase the viscosity of cytosol, and this indicates that under normal circumstances, there is temperature enables the enzymes to work perfectly and at the same time helps to provide a suitable viscosity which enables the cell to form the unstable area and will

be called the Crossed temperature. Crossed temperature or the critical temperature is the temperature which intersects the work of enzymes efficiently enough to accomplish an action with the formation of the unstable area and maintain enough activity to allow assemblage of actin units, as the increase in temperature above crossed temperature grade could lead to increase efficiency of one of the agents at the expense of the other. But in the experiment we want to prove to the existence of unstable area (keeping enzyme activity and time as constant), so we will alter temperature to reach a viscosity that will allow us to notice or gauge the pressure.

ACKNOWLEDGMENTS

I would like to say my Thank You to each and everyone of Dr: Pierre Schembri-Wismayer, D.S. Sheriff, Salem bozireda, Maher Edheba, Aisha Alaraibe, Mohamed Elgargouri, Ali Alkharm, Ez eldeen gasser, salha zobe and everyone who have read my research and helped me to share it around, and finally i would like to thank my family who has supported me all along my research for three years.

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