Intracellular pH as an electrostatic regulator of the spindle assembly checkpoint

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Abstract

Experiments have shown that intracellular pH of many cells rises to a maximum at the onset mitosis, subsequently decreasing 0.3 to 0.5 pH units by the end of mitosis. This result, and observations that tubulin net charge depends strongly on pH, may be significant for microtubule (MT) dynamics during mitosis. In vivo studies demonstrate that MT dynamics is sensitive to pH, with MT growth favored by higher pH values. Thus it seems likely that the shift from the dominance of microtubule growth during prophase, and to a lesser extent during prometaphase, to a parity between MT polymerization and depolymerization during metaphase chromosome oscillations is a consequence of a gradually decreasing intracellular pH during mitosis. A long-standing problem in the cell biology of mitosis concerns the operation of the spindle assembly checkpoint, a surveillance mechanism that delays anaphase-A onset until all chromosomes are attached to the spindle. When improper chromosome attachments persist into anaphase, chromosome segregation is defective and cells containing abnormal numbers of chromosomes can result leading to genetic diseases such as cancer. Here we propose that a consequence of relatively high intracellular pH near the metaphase-anaphase transition results in an anaphase-A delay that functions as the spindle assembly checkpoint.

Introduction

Cell cycle checkpoints ensure that a previous phase is complete before a cell is permitted to move forward. Some of the difficulties in interpreting experimental results regarding checkpoints can be attributed to a failure to adhere to definitions. For example, ascribing delays into anaphase-A to "activation of the spindle assembly checkpoint" misrepresents the situation since checkpoints are defined as being active as a cell starts mitosis. There is also a disagreement between whether the "wait anaphase (spindle assembly checkpoint) signal" involves defects in the forces associated with spindle-bound kinetochores, or the absence of spindle-kinetochore attachments. Finally, the separation of sister chromatids produces a state that a short time before would have prevented progression into anaphase. This is because the splitting of sister chromatids releases kinetochore tension, but the spindle assembly checkpoint machinery does not respond (see below). Although several recent studies [1-3] have made some inroads into resolving this anomaly, a number of basic questions still remain [4].

We propose here a "global" mechanism for the spindle assembly checkpoint based on a changing intracellular pH that appears to resolve these inconsistencies.

Some Cellular Electrostatics

In the cytoplasmic medium (cytosol) within biological cells, it has been generally thought that electrostatic fields are subject to strong attenuation by screening with oppositely charged ions (counterion, or Debye screening), decreasing exponentially to much smaller values over a distance of several Debye lengths. The Debye length within cells is typically given to be of order 1 nm, and since eukaryotic cells have much larger dimensions, one is tempted to conclude that electrostatics is not a major factor in explaining mitotic chromosome movements. However the presence of microtubules, as well as other factors discussed below, force this assumption to be reconsidered.

The characteristics of microtubule lengthening (polymerization) and shortening (depolymerization) follow a pattern known as "dynamic instability": that is, at any given instant some microtubules are growing, while others are undergoing rapid breakdown. In general, the rate at which microtubules undergo net assembly or disassembly varies with mitotic stage [5]. Changes in microtubule dynamics are integral to changes in the motions of chromosomes during mitotic stages. Poleward and antipoleward chromo-some motions occur during prometaphase and metaphase. Antipoleward motions dominate during the congressional movement of chromosomes to the cell equator region, and poleward motion prevails during anaphase-A. It is assumed here that poleward chromosome motions are in response to disassembling kinetochore microtubules at kinetochores and poles, and argued elsewhere [6], that antipoleward chromosome motions are best explained by assembling microtubules at chromosome arms.

Experiments have shown that intracellular pH (pH_i) of many cells rises to a maximum at the onset of mitosis, subsequently falling steadily through cell division. Although it is experimentally difficult to resolve the precise starting time for the decrease in pH_i during the cell cyle, it appears to drop 0.3 to 0.5 pH units from typical peak values of 7.3 to 7.5 measured during prophase when microtubule polymerization is favored [7,8].

Studies have shown that in vivo microtubule polymerization is favored by higher pH values [9], in contrast with in vitro studies which suggest a pH optimum in the range of 6.3 to 6.9. The disagreement between these values

has been considered in relation to the nucleation potential of microtubule organizing centers like centrosomes [9], suggesting that pH_i regulates the nucleation potential of microtubule organizing centers [10, 11]. Experiments have also shown that ionic concentrations play an important role in microtubule polymerization [12]. Taken together, these observations seem to favor the more complex physiology of in vivo analyses to resolve this question.

Cellular electrostatics is strongly influenced by significantly reduced counterion screening due to layered water adhering to charged molecules. Such water layering with consequent reduction or elimination of Debye screening at charged proteins has long been theorized [13, 14] and has been confirmed experimentally [15]. Additionally, water between charged proteins at close separations (see below) has a dielectric permittivity that is considerably reduced from the bulk value far from charged surfaces [16-18]. The combination of these two effects (or conditions) water layering and reduced dielectric constant can significantly influence cellular electrostatics in a number of important ways. This is especially true in relation to mitosis.

A number of investigations have focused on the electrostatic properties of microtubule tubulin subunits [19-22]. Large scale calculations of tubulin have been conducted using molecular dynamics programs along with protein parameter sets [23]. The dipole moment of tubulin has been calculated to be as large as 1800 Debye units [20, 24]. Studies have shown that tubulin net charge depends strongly on pH, varying quite linearly from 12 to 28 (electron charges) between pH 5.5 and 8.0 [24, 25]. This could be important for microtubule dynamics during mitosis because, as noted above, many cell types exhibit a decrease of 0.3 to 0.5 pH units during mitosis.

Kinetochore molecules self-assemble onto highly condensed, negatively charged DNA at centromeres [26], indicating that kinetochores may exhibit positive charge [6, 17]. This is an example of an important aspect of electrostatic interactions within cells: namely their longer range compared to other intracellular molecular interactions, with the resulting capacity of electrostatic force to organize molecules and structures within cells. Evidence for positive charge at kinetochores also comes from the presence of highly basic kinetochore molecules in the Dam1 complex. In particular, the isoelectric points of Dam1p, Duo1p, and Spc34p are 9.97, 10.76, and 8.6, respectively. Significantly, experiments have revealed that the microtubule binding module of the Dam1 complex involves these three molecules; acidic proteins Ask1p, Spc19p, and Dad2p fail to bind to micro-tubules [27].

Intracellular pH As A Clock For Mitosis

Tubulin has a large overall negative charge of -20 at pH 7, and as much as 40 % of the charge resides on the C-termini, which extend outward from the microtubule axis as a function of pH_i, (e.g., 4–5 nm at pH_i 7 [23]). It therefore seems likely that increased tubulin charge and the resulting greater extension of C-termini may be integral to an increased probability for microtubule assembly during prophase when pH_i is highest [8]. This self-assembly would be aided by (1) reduced counterion screening due to layered water, and (2) the reduced dielectric constant be-tween charged protein surfaces. These two effects (or conditions) likely increase the efficiency of microtubule self-assembly in asters and spindles during prophase by (1) allowing electrostatic interactions over significantly greater distances than counterion screening dictates, and (2) further increasing the strength of these interactions by an order of magnitude due to an order of magnitude reduction in the cytosolic dielectric constant between charged

protein surfaces separated by critical distances [18]. For brevity, separations of 0 to 3 nm (and, due to the reduced dielectric constant between charged molecular surfaces, 1 to 2 nm beyond) between charged surfaces will be designated as critical distances/gaps. An electrostatic component to the biochemistry of the microtubules in assembling asters is consistent with experimental observations of pH effects on microtubule assembly [9], as well as the sensitivity of microtubule stability to calcium ion concentrations [28, 29].

Thus over the critical distances consistent with a reduced dielectric constant and modified counterion screening, it is probable that the net (so called monopole) charge, as well as the electric dipole nature of tubulin dimers would allow tubulin dimer microtubule subunits to align laterally and end-to-end, facilitating the formation of asters and mitotic spindles during elevated pH_i prophase conditions [30, 31].

In addition to addressing force generation for post-attachment chromosome motions [6,17,18], a cellular electrostatics approach to mitotic motions can also account for the timing and sequencing of the detailed changes in these motions. These can be attributed to changes in microtubule dynamics based on a progressively increasing microtubule disassembly to assembly (disassembly/assembly) probability ratio for kinetochore microtubules that is caused by a steadily decreasing pH_i during mitosis [32]. An increased probability for microtubule depolymerization, as compared to the prophase predominance of microtubule assembly, is consistent with experimental observations of alternating poleward and antipoleward motions-with antipoleward motions more probable-of monovalently attached chromosomes during prometaphase.

As discussed elsewhere [33], after a bivalent attachment to both poles, electrostatic poleward forces toward both poles acting in conjunction with inverse square antipoleward forces exerted between negatively charged microtubule free plus ends and negatively charged chromosome arms explains chromosome congression. Metaphase chromosome midcell oscillations are indirect experimental evidence for a microtubule disassembly/assembly probability ratio approaching unity as pH_i continues to decline.

At late metaphase, before anaphase-A, experiments reveal that poleward disassembly forces acting at kinetochores and poles stretch the intervening centromeric chromatin, producing high kinetochore tensions [34]. These high tensions are attributed here to a sustained microtubule disassembly to assembly ratio increase caused by a continuously lowering pH_i. A lower pH_i would also elevate the expression of positive charge on kinetochores. The resulting greater repulsive force between sister kinetochores acting in combination with increased microtubule disassembly force tension is integral to an explanation of the spindle assembly checkpoint, as well as chromatid separation.

Thus regarding post-attachment chromosome motions through metaphase, it seems reasonable to ascribe an increasing dissassembly to assembly probability ratio-with attendant changes in microtubule dynamics and associated mitotic chromosome motions through metaphase-to an experimentally observed steadily decreasing pH_i. We may then envision a decrease in pH_i from a peak at prophase favoring microtubule assembly, declining through prometaphase, and continuing to decline through metaphase when parity between microtubule assembly and disassembly leads to midcell chromatid pair oscillations, culminating in increased microtubule disassembly-associated kinetochore tension late in metaphase, as the cell's

master clock controlling microtubule dynamics, and consequently the events of mitosis [32].

One might also be tempted to attribute the more complete dominance of microtubule disassembly with an accompanying predominance of poleward disassembly forces at kinetochores and poles during anaphase-A to a continued decrease in intracellular pH. However, as discussed elsewhere [6,17], any additional lowering of pH_i after metaphase may work in conjunction with increased calcium ion concentration (Ca^{2+}) [35, 36] as major determinants of anaphase-A motion.

Revisiting the Spindle Assembly Checkpoint

An understanding of the operation of the spindle assembly checkpoint has been elusive. We propose here that spindle assembly checkpoint behavior may be explained within the context of the present work as the result of a continuing elevated pH_i near the metaphase-anaphase transition. A higher pH_i would keep the microtubule disassembly to assembly probability ratio from increasing through metaphase, and would also reduce the manifestation of increased positive charge on sister kinetochores, with both outcomes serving to curtail the buildup of kinetochore tension. This tension is necessary for stabilizing proper (amphitelic) chromosome attachments due to the increased distance between sister kinetochores, with the resulting spatial separation of Aurora-B from its substrates [37]. This is because whenever kinetochores are not under tension, the phosphorylation action of Aurora-B is active, the kinetochore releases microtubules, and proper microtubule attachments are destabilized [38]. Because centromeres of improper (merotelic or syntelic) attachments are not stretched by increased tension, erroneous microtubule attachments to the spindle are destabilized under either low or high tension conditions. Both proper and improper chromosome attachments will be fragile under low tension conditions when it is also likely that insufficient force for chromatid separation is present. In addition, a decreased microtubule disassembly to assembly probability ratio would result in more stable microtubules, a condition that is counter-productive to error correction mechanisms, as demonstrated recently [39].

Thus we have (1) an increased probability of erroneous attachments due to a diminished efficiency for correcting mistakes [40] resulting from a reduced microtubule deploy-merization rate, (2) decreased kinetochore tension resulting in a destabilization of proper chromosome attachments [41], and (3) insufficient force for initial separation of chromatids, with all three outcomes due to a higher pH_i near the metaphase-anaphase transition.

Thus anaphase-A may be delayed by conditions that are due to a lingering high intracellular pH at late metaphase. Ultimately, a sufficiently decreased pH_i will obviate the above three conditions, and initiate anaphase. Since it is likely that all chromosomes will then be attached to the spindle as a result of the (delayed) lower pH_i, there will be the appearance of a spindle assembly checkpoint.

Conclusions

High pH_i during prophase favors spindle assembly. This involves greater electrostatic attractive forces between tubulin dimers. Due to reduced counterion screening and the low dielectric constant of layered water adhering to charged tubulin dimers, the necessary attraction and alignment of tubulin dimers during spindle self-assembly would be enhanced by the considerably increased range and strength of electrostatic attractions

between oppositely charged regions.

Changes in microtubule dynamics are integral to changes in chromosome motions during mitosis, and can be attributed to an associated change in intracellular pH (pH_i), a logical experimental target for identifying the underlying factors that control mitotic events throughout the cell cycle. In particular, a decrease in pH_i through mitosis may act as a master clock controlling microtubule disassembly to assembly probability ratios by altering the electrostatic interactions of tubulin dimers. This, in turn, would determine the timing and dynamics of post-attachment mitotic chromosome motions through metaphase.

The shift from the dominance of microtubule growth during prophase, to a lesser extent during prometaphase, and to approximate parity between microtubule polymerization and depolymerization during metaphase chromosome oscillations, culminating in increased kinetochore tension just prior to anaphase, can be attributed to a gradual downward pH_i shift during mitosis.

Failure of cells to achieve a sufficiently low pH_i at the metaphase-anaphase transition leads to conditions that mimic the operation of a spindle assembly checkpoint. A delayed decrease in pH_i ultimately allows progression to anaphase.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

LJG conceptualized the theoretical aspects of this article and DHS provided intellectual contributions. Both authors read and approved the final manuscript.

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