Journal of Theoretical Biology 407 (2016) 143–154

Contents lists available at ScienceDirect

Journal of Theoretical Biology

journal homepage: www.elsevier.com/locate/yjtbi

Stochastic modelling suggests that an elevated superoxide anion - hydrogen peroxide ratio can drive extravascular phagocyte transmigration by lamellipodium formation

Siddhartha Kundu [a,b,c,*,1]

[a] Department of Biochemistry, Dr. Baba Saheb Ambedkar Medical College & Hospital, Government of NCT Delhi, Sector – 6, Rohini, Delhi 110085, India
[b] Mathematical and Computational Biology, Information Technology Research Academy (ITRA), Media Lab Asia, 2nd Floor, Block 2, C-DOT Campus, Mehrauli, New Delhi 110030, India
[c] School of Computational and Integrative Sciences, Jawaharlal Nehru University, New Mehrauli Road, New Delhi 110067, India

HIGHLIGHTS

- Aggregates of higher order RRA-oligomers may facilitate lamellipodium formation.
- RRA-oligomer driven free radical accumulation can prolong membrane perturbation.
- The response curve of ECSOD is bimodal, and is separated by a steady-state phase.
- There is an inverse association between superoxide anions and hydrogen peroxide.

ARTICLE INFO

Article history:
Received 3 May 2016
Accepted 1 July 2016
Available online 2 July 2016

Keywords:
Actin
Chemotaxis
Free radicals
Lipid raft
Stochastic model

ABSTRACT

Chemotaxis, integrates diverse intra- and inter-cellular molecular processes into a purposeful patho-physiological response; the operatic rules of which, remain speculative. Here, I surmise, that superoxide anion induced directional motility, in a responding cell, results from a quasi pathway between the stimulus, surrounding interstitium, and its biochemical repertoire. The epochal event in the mounting of an inflammatory response, is the extravascular transmigration of a phagocyte competent cell towards the site of injury, secondary to the development of a lamellipodium. This stochastic-to-markovian process conversion, is initiated by the cytosolic-ROS of the damaged cell, but is maintained by the inverse association of a *de novo* generated pool of self-sustaining superoxide anions and sub-critical hydrogen peroxide levels. Whilst, the exponential rise of $O_2^{\cdot-}$ is secondary to the focal accumulation of higher order lipid raft-Rac1/2-actin oligomers; $O_2^{\cdot-}$ mediated inactivation and redistribution of ECSOD, accounts for the minimal concentration of H_2O_2 that the phagocyte experiences. The net result of this reciprocal association between ROS/ RNS members, is the prolonged perturbation and remodeling of the cytoskeleton and plasma membrane, a prelude to chemotactic migration. The manuscript also describes the significance of stochastic modeling, in the testing of plausible molecular hypotheses of observable phenomena in complex biological systems.

1. Introduction

The chemically driven motion of a cell (chemotaxis), is the migration of cells along a concentration gradient of a particular chemical moiety. This conserved evolutionary phenomenon is commonly seen in endothelia, neutrophils and monocyte-macrophages of higher organisms, as well as, in simple eukaryotes (*Dictyostelium discoideum*) and prokaryotes. The chemical structure notwithstanding, inductive behavior in a cell, for a particular compound, depends on a dynamic clustering of a multitude of factors. The proposed mechanisms involve flagellar clockwise

Abbreviations: DSM, Dynamic Stochastic Model; ECSOD, Extra-Cellular Superoxide Dismutase; PMN, Polymorphonuclear Leukocyte; ROS, Reactive Oxygen Species; RNS, Reactive Nitrogen Species; RRA, Raft-Rac1/2-Actin; SO, Superoxide Anions; SSM, Static Stochastic Model

* Correspondence address: Department of Biochemistry, Dr. Baba Saheb Ambedkar Medical College & Hospital, Government of NCT Delhi, Sector – 6, Rohini, Delhi 110085, India.
E-mail address: siddhartha_kundu@yahoo.co.in

[1] Media Lab Asia: A section 25 not-for-profit organization of the Department of Electronics and Information Technology, Ministry of Communications and Information Technology- Government of India.

http://dx.doi.org/10.1016/j.jtbi.2016.07.002
0022-5193/

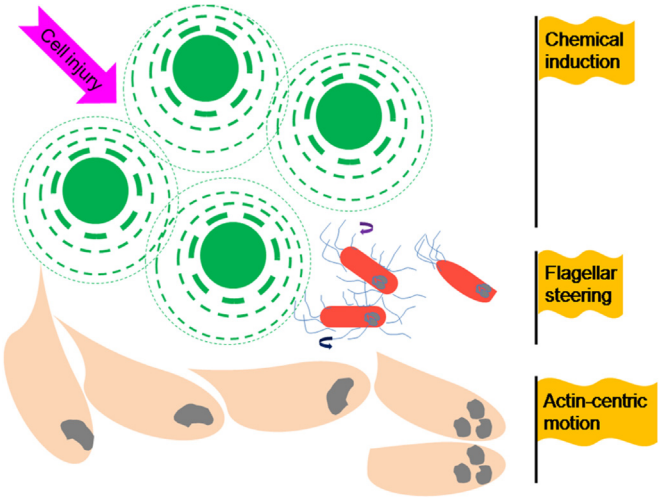

Fig. 1. Chemotactic motility in diverse organisms. The movement of cells towards a chemical moiety is as diverse as the organisms themselves and includes: flagellar rotation, pseudopodia generation, and amoeboid motion. Both, attractant and repellant mobility have been demonstrated under laboratory conditions. The inflammatory response in tissues is spatio-temporal with the release of damaging free radicals or their precursors secondary to cellular injury, increased vascular permeability, diapedesis, and transmigration of phagocytic competent cells towards a chemo-attractant(s).

(CW) and counter-clockwise (CCW) motions, coordinated microspike and bleb development (*D. discoideum*), and F-actin mediated pseudopodia generation (Fig. 1) (Yousif et al., 2015; Lin et al., 2015; Tyson et al., 2014; Brown et al., 2002). The resultant movement may either be convergent (chemoattractant) or divergent (chemorepellant). In *Escherichia coli*, the chemical nature of the inducer determines the quanta of movement. Attractants include: serine, aspartic acid, and glucose, while repellants such as fatty acids constitute the noxious component of the stimulus (Edgington and Tindall, 2015; Nagy et al., 2015; Pasupuleti et al., 2014; Danielson et al., 1994). These effects are mediated by extensive signal transduction networks, downstream of at least five transmembrane receptors (Shimaoka et al., 2004; Borkovich et al., 1989). In higher organisms, the progesterone secreted by the cumulus oophorous influences spermatozoal movement when in proximity with the ovum (Guidobaldi et al., 2008). The social amoeba, *D. discoideum*, exhibits a trimodal life cycle. The transition from the stage of nutritional deprivation to one of surplus, results in a shift from unicellular morphology to an elaborate multicellular sessile fruiting body. This is facilitated by an intermediate aggregate form referred to as the 'slug'. Chemotaxis, is exhibited early in the lifecycle with the stimuli being folic acid (FA) and cyclic-adenosine monophosphate (cAMP) (Wessels et al., 2014; Srinivasan et al., 2013; Segota et al., 2013).

Inflammation, an innate immune reaction to noxious stimuli (infection, injury), is initiated when the first line of host defense is breached, *i.e.*, a compromise in the integrity of anatomical- (skin and acidic pH of the gastric mucosa) and physiological-barriers. This generic patho-physiological response is characterized by: local hyperemia and raised skin temperature (secondary to chemical vasodilation), swelling (exudation of fluid into the interstitial space through endothelial gap widening), pain (involvement of nerve endings), and tissue damage (phagocytosis following diapediasis, extravasation, and transmigration). The inflammatory process, within tissues, maybe secondary to a range of causal irritants, *viz.*, physical, infective, chemical. These covert occurrences, however, are not entirely benign and are often precursors to a long term systemic pathology (dysplasia and squamous cell carcinoma; atherosclerosis and cardiovascular disease; inflammatory bowel disease; hepato-biliary pathology as in steatosis, hepatitis, cirrhosis, and hepatocarcinoma). Mediators of inflammation could be chemokines, free radicals, an acid/base environment, among several others. Reactive oxygen species (ROS), are a molecular ensemble of free radicals and metabolic intermediates and comprise superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\cdot OH$) and hydroxide ions (OH^-). These occur in tandem with the reactive nitrogen species (RNS) of $ONOO^-$, NO^-, and NO_2. ROS, as signaling molecules regulate the balance between cellular proliferation and senescence, influence the magnitude of immune response, and participate in cytoskeleton remodeling and migration (Forman et al., 2004; Torres and Forman, 2003; Mikkelsen and Wardman, 2003). The concentration of free radicals in biological systems is dependent on the interleaved processes of initiation, propagation, and termination. The principal sources of ROS are enzymes either as a primary product (Xanthine oxidase, EC 1.17.3.2; NADP(H) oxidase, EC 1.6.3.1; Nitric oxide synthase, EC 1.14.13.39) or secondary to diffusion from the active site (2-oxoglutarate dependent dioxygenases, EC1.14.11.x) (Kundu, 2015a, 2015b; Rocklin et al., 2004). The pathways that contribute to maintaining this chain reaction result in the generation of substrate radicals, and include the hydro- and endo-peroxides of unsaturated membrane lipids (Lipoxygenases, EC 1.13.11.x) or hypohalous acid (Myeloperoxidase, EC 1.11.2.2). Terminators of this cascade include dismutation, either enzymatic (Superoxide dismutase, EC 1.15.1.1; Catalase, EC 1.11.1.6; Glutathione peroxidase, EC 1.11.1.9) or spontaneous self-association; small molecule scavenging (ascorbic acid, urea, retinol, tocopherols), and intracellular transport by the chloride anion transporter-3 (ClC3) (Fisher, 2009; Hawkins et al., 2007).

In the absence of an overt ciliary or flagellar contributory influence, the cytoskeletal network in cells remains a watershed of subtle chemical fluctuations that transpire extracellularly. Migration may be in response to a gradient of morphogens (embryonic tissue patterning), or to mitigate the effects of a noxious stimulus (neutrophils, monocyte-macrophages). The signal transduction route usually involves GTP-binding protein(s), secondary messengers, and several cycles of kinase-phosphatase activity (Sadhu et al., 2003; Sakai et al., 2003; Sastry et al., 2002; Zervas et al., 2001; Angers-Loustau et al., 1999; Tamura et al., 1998; Allen et al., 1997; Nobes and Hall, 1995; Chen and Guan, 1994). Monomeric GTPase (Rac, Rho, and Cdc42) driven motility is accomplished by extending lamellipodia at a leading edge, with a concomitant retraction of the lagging end (Wong et al., 2006; Van Keymeulen et al., 2006; Tzima, 2006; Watanabe et al., 2004; Fukata et al., 2003; Allen et al., 1997). Since the formation of membrane bound cytoplasmic extensions is, inherently stochastic, with several extrusions developing in parallel, the final outcome may be a function of a molecular filter that may function to sense and modulate the chemically defined signaling gradient (Kundu and Subodh, 2011; Hattori et al., 2010; Andrew and Insall, 2007). The output of this hypothetical unit could then initiate the appropriate feedback mechanism(s) by actuating downstream pathways, thereby, ensuring the maturation of a single dominant extension with consequent vectorial movement. A role, *albeit*, indirect, for ROS/RNS in this cell steering activity has been postulated and investigated (Kundu and Subodh, 2011; Hattori et al., 2010; Andrew and Insall, 2007). The emphasis of much of this work was on the hydrogen peroxide mediated activation of the GTPase-PLC/D-calcium/PTEN or Axl-PI3K-Akt1 transduction axes to bring about migration secondary to redox-based cytoskeletal rearrangement (Huang et al., 2013; Andrew and Insall, 2007; Fischer et al., 2005; Usatyuk et al., 2003; Vepa et al., 1999; Hastie et al., 1998; Natarajan et al., 1996). However, H_2O_2 is a potent phosphatase inhibitor, and can rapidly increase the pool of kinase-mediated phophorylated proteins. This phosphate-sink, can deplete the cell of available ATP, saturate the