Available online at www.sciencedirect.com
ScienceDirect
Cellular Signalling 20 (2008) 1415–1422
CELLULAR SIGNALLING
www.elsevier.com/locate/cellsig

Review

Highlighting the role of Ras and Rap during *Dictyostelium* chemotaxis

Arjan Kortholt, Peter J.M. van Haastert *

Department of Molecular Cell Biology, University of Groningen, Kerklaan 30, 9751NN Haren, The Netherlands

Received 11 January 2008; accepted 6 February 2008
Available online 1 April 2008

Abstract

Chemotaxis, the directional movement towards a chemical compound, is an essential property of many cells and has been linked to the development and progression of many diseases. Eukaryotic chemotaxis is a complex process involving gradient sensing, cell polarity, remodelling of the cytoskeleton and signal relay. Recent studies in the model organism *Dictyostelium discoideum* have shown that chemotaxis does not depend on a single molecular mechanism, but rather depends on several interconnecting pathways. Surprisingly, small G-proteins appear to play essential roles in all these pathways. This review will summarize the role of small G-proteins in *Dictyostelium*, particularly highlighting the function of the Ras subfamily in chemotaxis.

Keywords: *Dictyostelium*; Chemotaxis; Ras; Rap; Guanine exchange factor; GTP-binding protein

Contents

1. Introduction

Chemotaxis or directional movement towards a chemical compound is an essential property of many cells [1]. For example, chemotaxis is critical for the sourcing of nutrients by prokaryotes, the organization of the embryo in metazoa, the formation of multicellular structures in protozoa and the migration of lymphocytes during immune response [2–5]. Chemotaxis is also linked to the development and progression of many diseases including asthma, arthritis, arteriosclerosis, and cancers [6–8]. Therefore further insight into the molecular mechanism of chemotaxis is important for the understanding of many biological processes.

Research on eukaryotic chemotaxis has progressed substantially, mainly through the study of neutrophils or the amoeba *Dictyostelium discoideum* as model systems [1]. *Dictyostelium* is a free-living soil amoeba that feeds on bacteria. They chase bacteria by chemotaxing towards folic acid, which is secreted by the bacteria. Upon starvation cells undergo a tightly regulated developmental process. During development single cells undergo a drastic change in gene expression and start to secrete cAMP. Neighbouring cells respond by migrating toward the chemoattractant cAMP and by secreting cAMP themselves, resulting in multicellular fruiting bodies. Recently the assembly of the *Dictyostelium* genome was completed [9]. The 34 Mb

* Corresponding author. Tel.: +31 503634172; fax: +31 503634165.
E-mail address: P.J.M.van.Haastert@rug.nl (P.J.M. van Haastert).

0898-6568/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellsig.2008.02.006

genome contains many genes that are homologous to those in higher eukaryotes and are missing in other lower eukaryotes such as yeast [9]. As the mechanism of chemotaxis is essentially identical in all eukaryotes, *Dictyostelium* offers a genetically tractable model in which to study chemotaxis [1,10].

Chemotaxis is a complex cellular process involving a multitude of signalling pathways and molecules. Eukaryotic chemotaxis involves three distinct and separable processes: directional sensing, cellular motility and cell polarity [1,11]. The first step in directional sensing is the binding of the chemoattractant to cell-surface G-protein coupled receptors (GPCRs), which are characterized by seven membrane-spanning α-helices. Upon ligand binding, GPCRs undergo a conformational change that enables them to activate heterotrimeric G-protein; by exchanging the G-protein bound GDP to GTP. This exchange promotes dissociation of the three subunits as Gα-GTP and a Gβγ dimer, both of which can regulate a diverse set of downstream effectors. Due to the intrinsic Gα associated GTPase activity, GTP is hydrolysed to GDP, and the inactive heterotrimer is formed again [12–14].

In *Dictyostelium* four cAMP receptors have been identified (cAR1–cAR4). cAR1–4 are expressed sequentially throughout development and can all support chemotaxis. Since the types of cAMP receptor expressed sequentially during development have decreasing affinities, they are able to cope with an increase in extracellular cAMP concentration during the aggregation stage [15,16]. cAR2–4 have a relative low affinity for cAMP and are important during multicellular stage, whereas cAR1 has a high affinity for cAMP and is essential for signal transduction during early development and chemotaxis. *Dictyostelium* contains one G-protein β subunit and one Gγ subunit, which are both essential for chemotaxis. Of the 11 identified Gα subunits, Gα2 is coupled to the cAR1 receptor and most important for cAMP-mediated chemotaxis [17–19], Gα1 is coupled to a yet unidentified CMF receptor [20] and to cAR1 leading to inhibition of PLC activity [21], while Gα4 is most likely coupled to a yet unidentified receptors for folate [20,22]. Disruption of Gα2 results in cells that are unable to aggregate and do not respond to cAMP stimulation [18], a phenotype similar to strains deleted in cAR1 and Gβ [18,23,24], these phenotypes indicate that their function is essential for directional sensing and chemotaxis. Surprisingly, detailed analysis of the localisation and dissociation of these upstream components of the chemotactic signal-transduction machinery has revealed that cell polarization is established downstream of G-proteins. In cells exposed to a cAMP gradient, cAR1 is expressed uniformly on the plasma membrane [25], as are the G-protein subunits [26,27], while G-protein activation reflects the shallow external gradient of cAMP [26]. Subsequently the activated G-proteins convert the signal into the interior of the cell where they activate a complex network of signalling molecules, resulting in a gradient of cellular components. This gradient induces coordinated remodelling of the cytoskeleton and cell adhesion to the substratum, which leads to formation of new actin filaments in the front that induce the formation or stabilization of local pseudopodia, and acto-myosin filaments that inhibit pseudopod formation and retract the uropod. The final outcome is cellular movement up the chemoattractant gradient.

An important response in both the establishment of cell polarity and chemotaxis is the formation and accumulation of phosphatidylinositol-3,4,5-triphosphate ($PI(3,4,5)P_3$) at the leading edge [28–30]. Phosphoinositide 3-kinase (PI3K) produces $PI(3,4,5)P_3$ by phosphorylation of "$PI(4,5)P_2$", and phosphatase and tensin homologue deleted on chromosome ten (PTEN) converts $PI(3,4,5)P_3$ back to $PI(4,5)P_2$. Upon stimulation PI3K is rapidly translocated and activated at the leading edge, while PTEN is delocalised from the front to the lateral and posterior membrane [28–30]. By this reciprocal temporal and spatial regulation of PI3K and PTEN a $PI(3,4,5)P_3$ gradient is accomplished. The accumulation of $PI(3,4,5)P_3$ at the leading edge results in recruitment of $PI(3,4,5)P_3$ binding molecules and subsequent pseudopod extension [28,30,31]. Although, initial studies described an essential role for PI3K, recent studies have shown that chemotaxis does not depend on the $PI(3,4,5)P_3$ pathway [32–34]. Cells with disruption in all five genes encoding type I PI3Ks still exhibit normal chemotaxis, suggesting that PI $(3,4,5)P_3$ mediated signalling works in conjunction with one or more pathways to mediate chemotaxis [33]. Two studies identified phospholipaseA2 as a chemotactic pathways parallel to the PI3K pathway [32,34]. Chen et al. described that loss of the Ca^{2+}-independent phospholipaseA2 (iPLA2) homologue *plaA* results in cells that are hypersensitive to the PI3K inhibitor LY294002 and show dramatically reduced chemotaxis. Consistently, van Haastert et al. showed that inhibition of either PI3K or PLA2 has minor effects on chemotaxis, whereas inhibition of both enzymes inhibits chemotaxis nearly completely. The effects of inhibition of PI3K and PLA2 on chemotaxis are less severe at later stages of development, suggesting the presence of a third pathway in longer starved cells. Genetic studies suggest that this third pathway consists of a soluble guanylyl cyclase [35]. These data show that chemotaxis does not depend on a single molecular mechanism but depends on several interconnecting pathways.

Many studies have focused on the signal pathways downstream of the heterotrimeric G-proteins. Several of these studies have revealed that an early event in chemotaxis is the activation of small G-proteins. In this review we will focus on small G-proteins in *Dictyostelium*, particularly highlighting the important role of the Ras subfamily in chemotaxis.

2. Small G-proteins

Small GTPases are monomeric GTP-binding proteins, which function as molecular switches to control a wide variety of cellular functions. They switch between an inactive GDP-bound and active GTP-bound state (Fig. 1). Ras activity is regulated by guanine nucleotide exchange factors (GEFs) that catalyse the exchange of GDP for GTP, thereby activating the Ras protein. GTPase activating proteins (GAPs) stimulate an otherwise low intrinsic GTPase activity by many orders of magnitude, reverting the conformation back to the inactive GDP-bound form [36]. The eukaryotic small GTPase superfamily can be divided into five major subfamilies: Ras, Rho, Rab, Ran and Arf [37]. In the genome of *D. discoideum* an unusual large amount of small GTPases were identified. It contains a total of 119 genes encoding small GTPases, encompassing all five subfamilies [9,38]. Proteins of the Rab, Ran and Arf subfamilies are mostly implicated in the regulation of vesicular or nuclear transport,