Electrostatic charge at the membrane surface has emerged as a crucial determinant of the localization and activation of many proteins containing polycationic domains in their amino acid sequence. The spatiotemporal regulation of surface charge, as well as the downstream effects of dysregulation of surface charge, may have a significant impact on many of the signaling molecules important to cancer biology such as K-ras. Cancer Res; 70(4): 1277–80. ©2010 AACR.

Surface Charge: A Historical Perspective

Surface charge is a distinct phenomenon from transmembrane potential, which is an electrodifusional voltage, created by differences in inorganic ion concentrations and permeabilities across the membrane. Surface charge, however, is determined by the cumulative effect of charged lipid headgroups within the membrane itself. Both cationic and anionic functional groups contained in the lipid headgroups contribute to the net electric field at membrane surfaces. In the plasma membrane, there is a relative predominance of anionic species in the inner leaflet (4–7), bestowing it with an overall negative electrostatic profile. It is the charge at the surface of the inner aspect of the plasma membrane that will be discussed further.

Two monovalent phospholipids, phosphatidylserine (PS) and phosphatidylserine (PS), and phosphatidylinositol (PI), make up the majority of the negative membrane surface charge. Although each of these molecules only contributes one negative charge unit to the membrane surface, PS and PI make up approximately 15% and 10% of the lipid content of the plasma membrane inner leaflet, respectively (7). This is in contrast to the zwitterionic lipid species PC and PE that, respectively, contribute 25% and 45% of the total plasma membrane lipid content. In addition to PS and PI, the polyvalent PI species PI(4)P, PI(4, 5)P2, and PS(3,4,5)P3 also contribute significantly to surface charge (1, 2). These PI species can be segregated into microdomains when bound by polycationic protein regions, increasing their local accumulation and influence on surface charge. The net effect of the relative accumulation of anionic phospholipids in the plasma membrane is an electric field of 10⁵ V/cm, capable of strongly attracting cationic proteins, peptides, and ions (8, 9).

The importance of membrane surface charge to protein targeting was first described by McLaughlin and colleagues. In a series of studies involving myristoylated alanine-rich protein kinase c substrate (MARCKS), they were able to show that both insertion of the myristoyl chain into the lipid bilayer and the electrostatic interaction of the polybasic domain of MARCKS with acidic phospholipids in the membrane were required for proper protein targeting (10). These observations built upon those made by Aderem, who showed that phosphorylation and dephosphorylation resulted in the cycling of MARCKS on and off the plasma membrane (11). Using large unilamellar vesicles (LUV), they showed that addition of 20% PS into a neutral vesicle increased the proportion of MARCKS peptide binding to the LUV by 100 fold (10). This interaction was disrupted by increasing the ionic strength of the assay solution, and by PKC-mediated phosphorylation of the MARCKS basic domain (10). Additionally, the presence of Ca²⁺/calmodulin, which...
binds the basic domain of MARCKS, decreased MARCKS binding to LUV containing acidic phospholipids (10). Taken together, these data supported the model put forward by McLaughlin and colleagues that the insertion of the myristol group into the membrane brought the basic domain into close proximity with the lipid bilayer, thereby increasing the chance of the basic domain binding to acidic phospholipids (10).

In subsequent studies, this model was refined to include the ability of MARCKS to electrostatically bind PI(4, 5)P2 and laterally sequester it within the plasma membrane inner leaflet, resulting in the reversible inhibition of PLC (12).

The dynamic nature of the electrostatic interaction between proteins and membrane phospholipids was shown by Silvius and colleagues, using a system whereby the presence of rapamycin causes heterodimerization of proteins containing the rapamycin-binding domain of mTOR (FRB) and FKBP12 (FKBP; ref. 13). A mitochondria-targeted chimera containing FKBP domains was coexpressed with various peptides based on the tail of H-ras or K-ras fused to FRB. Upon addition of rapamycin, these constructs heterodimerized at the mitochondria, provided the ras-containing chimera was able to dissociate from the plasma membrane (13). The H-ras tail, which includes a farnesylation signal and a "second signal" of two S-acylation sites, showed biphase translocation to the mitochondria, indicative of a more stable interaction at the plasma membrane (13). Interestingly, K-ras4B, which binds the plasma membrane using a polybasic domain, also exhibited dynamic association with the plasma membrane (13). The dynamic nature of K-ras4B membrane association provides it with the ability to "sample" the electrostatic environment of several membranes in order to function properly. Work by Tobias Meyer showed the importance of PI(3,4,5)P3 and PI(4, 5)P2 in plasmamembranous targeting of K-ras (1). Further, Choy and colleagues showed that whereas all CAAX motifs traffic to the endomembrane system prior to binding the plasma membrane, K-ras4B, with its polybasic domain, traverses a unique pathway en route to the plasmalemma (14). Although the greatest negative charge in the cell is at the plasma membrane inner leaflet, other sites are also negatively charged, albeit to a lesser degree. Yeung and Grinstein, using a novel PS probe, were able to show that PS is largely responsible for the intermediate level of negative charge found on endomembrane compartments (15, 16). Notably, when plasmalammal PI(4, 5)P2 is hydrolyzed, K-ras4B redistributes to less negatively charged, PS-containing endomembranes (15). This relocalization is facilitated by the dynamic nature of K-ras4B membrane association, and may play an important role in its ability to propagate signals from various intracellular locations. Further, these studies outline different roles for PI and PS species in establishing surface charge at specific sites of the endomembrane system.

The importance of the polybasic domain of K-ras came to the fore when Hancock and colleagues did fractionation experiments with H-ras and K-ras, expanding on the second signal hypothesis described above (17). Systematic replacement of the basic residues in K-ras with neutral ones resulted in decreased membrane association, providing real evidence for the requirement of both CAAX motif processing and electrostatic interactions for appropriate plasma membrane targeting (17).

The Regulation of Surface Charge

The above studies highlighted the impact that membrane surface charge has on cell signaling. It followed that changes in the membrane electrostatic potential could modify cell-signaling behavior. In general, there are two ways to regulate the effect of surface charge on protein targeting, shown schematically in Fig. 1: (1) modification of the net charge of the protein, or (2) alteration in membrane surface charge itself. Several proteins important in cancer biology are affected by surface charge, including K-ras, c-Src, and Rac1 (3). As shown by McLaughlin, phosphorylation of amino acids in a positively charged protein domain decreases the net charge of the protein, resulting in its dissociation from the membrane (10, 18). Additionally, interaction of the cationic protein with an anionic one, such as Ca2+/calmodulin, effectively shields the electrostatic interaction between the given protein domain and the plasma membrane (2, 10). The ability of phosphorylation to negatively regulate membrane localization of K-ras was shown by Bivona and colleagues, who reported that PKC-dependent phosphorylation of K-ras removed the protein from the plasmalemma (19). Further, the antineoplastic drug, bryostatin-1, was found to inhibit tumorigenesis in a K-ras phosphorylation-dependent manner, attaching clinical significance to the modulation of K-ras charge (19).

Modification of membrane surface charge through phospholipid metabolism also occurs, and mutations in PTEN or PI3K can potentially affect phosphoinositide homeostasis, in turn perturbing surface charge. PTEN is itself targeted to the membrane through electrostatic forces; polycationic stretches in its phosphatase and C2 domains are required for its plasma membrane localization (20). Phosphorylation at its C terminus shields the positive charge of PTEN, and cycling between phosphorylated and dephosphorylated states establishes an electrostatic switch that decisively modulates PTEN membrane association and thus its activation (20).

The spatial regulation of membrane electrostatics has emerged as an important factor in protein targeting. The advent of novel fluorescent probes for monitoring surface charge has allowed for the investigation of surface charge gradients throughout the endomembrane system. In phagocytic cells, negative surface charge diminishes along the endocytic pathway from nascent phagosome to lysosome (15, 16). This gradient is capable of affecting protein localization to the endosome. Current models of endosomal signaling platforms during receptor tyrosine kinase internalization are potential targets of this charge gradient; polycationic protein domains will have a decreasing attraction to the endosome membrane as it matures. This phenomenon has been shown by Yeung and colleagues (3, 15, 16) in macrophages: as surface charge decreases, proteins...
with fewer positive residues are recruited to the maturing phagosome. Similarly, as the PI-derived lipid content of the endosome changes, plasma membrane-localized signaling molecules with significant positive charge are released from the nascent phagosome (3). These findings could have profound implications for the development of endosomal signaling platforms, and are currently an area of active research.

**Surface Charge as a Determinant of Protein Localization**

The negatively charged membrane surface serves as a targeting motif for a variety of polybasic domain-containing signaling proteins, some of which are important in cancer biology. Using a genomic survey approach, Heo and colleagues identified 37 small GTPases of the Ras, Rho, Arf, and Rab protein families that target the plasma membrane through electrostatic interactions between C-terminal polybasic domains and the plasma membrane phosphoinositides PI(4, 5)P₂ and PI(3,4,5)P₃ (1). This group of proteins is exemplified by K-Ras, one of the most commonly mutated oncoprogic proteins in solid tumors. The K-Ras C terminus carries a net charge of +8 that is crucial for targeting the protein to the plasma membrane.

As described above for MARCKS and K-ras, polybasic domains are themselves insufficient to recruit proteins to their target membranes with few exceptions. Surface charge consequently functions as one arm of a two-component coincidence detection system whereby electrostatic interactions between anionic lipids and cationic amino acids work in tandem with protein lipid modification for correct protein localization.

In this system, the electrostatic interaction functions as a binary switch, not to be confused with reference to small GTPases as “molecular switches,” through which the generation and strength of membrane targeting can be regulated by changes in either the charge of the cationic domains or the anionic lipid binding surface, as detailed above (21). Through these mechanisms, the localization of signaling molecules, such as K-Ras, can be modulated by focal changes in surface charge (22, 23).

**Concluding Remarks**

Electrostatic phenomena have emerged as probable determinants of various cellular functions: membrane surface charge can target GTPases, compartmentalize signaling platforms, and provide specificity to signaling cascades. Many of these processes converge uniquely upon cancer biology; a large proportion of the proteins affected by surface charge, as well as several responsible for establishing surface charge, have previously been identified as tumor suppressors or oncogenes. Further, the failure of signaling...
cascade localization could be a critical step in the progression from normal cell to neoplasm. As the precise contribution of different phospholipids to membrane surface charge becomes apparent, manipulation of surface charge may become a viable strategy by which tumorigenesis may be controlled.

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No potential conflicts of interest were disclosed.

References


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Neil M. Goldenberg and Benjamin E. Steinberg

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