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Editorial

Pathology Hiding in Plain Sight: The *NF1* Plasma Membrane

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The passive voice and the active voice: for example, is it a matter of "at" or "to?" The *NF1* gene product, neurofibromin (Nfn), in its interaction with the oncoprotein, Ras, does something to the cell's plasma membrane (PM); the reactions in question do not merely happen at the cell's PM. Moreover, what the Nfn does to the PM in *NF1* cells has been seriously underestimated because of yet another perspective issue. The vast majority of publications on the membrane-bound interaction of Nfn and Ras have been from the perspective that, first and foremost – if not exclusively – the *NF1* gene is considered a "tumor suppressor gene." This is rather narrow, respecting the ancient origin and very highly conserved nature of the *NF1* alphabetical code, and the intuitive understanding that the vast majority of eukaryotic species are not at risk for tumors or cancer.

However, with the advent and ready availability of genomic analyses now being exploited to understand the nature of normal and abnormal primitive organisms, such as amoebae, Nfn has been identified as a very important contributor to ordinary cell biology, particularly as relates to nutrition and feeding. Fortunately, with the basic science work from the United Kingdom[1] and Southern California[2] and elsewhere, a more realistic picture of the *NF1* supergene[3] is coming to light. Bloomfield et al[1] and Zhang et al[2] have made it unequivocally clear that both the wild-type and mutant alleles at the *NF1* locus in amoeba – *Dictyostelium* specifically – do something to the organism's anatomic engagement with the rest of the world, that is, it's PM. Whether and how the organism lives depends on what its *NF1* gene does to its PM. This is a far cry from the *NF1* gene being exclusively a safeguard against cancer. The activity of Nfn is not merely apparent at the PM. The activity of Nfn is a key determinant of the nature and function of the PM. This fact is likely to be relevant to therapeutic strategies for *NF1*: attention to the gene's *raison d'être* would seem to be important.

According to some authors, the only purpose of the Ras "oncoprotein" is to be a – if not the – critical determinant of rapidly accelerated fibrosarcoma (Raf) activation and thereby the key regulator of the MEK, mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK) cascade and their consequent regulatory networks.[4-9] And almost all of the relevant literature regarding the physiological role and importance of Ras respects and amplifies this prejudice. Respecting that the over-riding concern of some researchers is the development of legitimate cancer treatments, we do, indeed, need to consider these details. One potential over-simplification: I'm using the word, "Ras," generically, essentially ignoring ultimately relevant differences between the several types of Ras, including those well-known – H-Ras, K-Ras, N-Ras – and the more esoteric ones – M-Ras, S-Ras, S_2-Ras.

Ras is docked at the PM, anticipating the extracellular triggers that impinge on the PM, ordinarily at specific effector receptors (e.g., the epidermal growth factor receptor, (EGFR)). As a naked, unadorned protein, Ras has no biological impact. In its active state, Ras is coupled to guanine triphosphate (GTP). When the GTP γ-phosphate has been removed/transferred, Ras is thereby coupled with guanine diphosphate (GDP). The dephosphorylation can be accounted for by Ras' weak intrinsic phosphatase (GTPase) catalysis, or by an extrinsic GTPase activating protein (GAP), ordinarily either the p120GAP or Nfn. Ras can then be reactivated by replacing the GDP with GTP *via* the guanine exchange factor (GEF). Attached to the PM, Ras, whether as the tri- or di-nucleotide is consistently attached to GEF and occasionally attached to Nfn. I will refer to the triplet agglomerations as GRTN (GEF-RasGTP-Nfn) or GRDN (GEF-RasGDP-Nfn).

How the PM-bound GEF-RasGTP pair is joined with Nfn to create the GRTN triplet involves yet another substance,

SPRED. (There are actually four SPRED moieties, numbered 1, 2, 3 or 4. For simplicity, I will use *SPRED1*, coded for by the *SPRED1* gene, which, when mutated, gives rise to the Legius syndrome.) Wildtype *SPRED1* is responsible for transporting cytosolic Nfn (Nfn^{C}) to the PM, and affording its attachment to RasGTP, completing the triplet GRTN, that is, $GRTN^{PM}$. Once this triplet is formed, SPRED molecules do not ordinarily contribute to $GRTN^{PM}$ dynamics, at least in terms of Raf activation, although we still have a lot to learn about $GRTN^{PM}$ control of PM dynamics themselves.

Inactive cytosolic Raf (Raf^{Ci}) occupies the vicinity near PM-attached GRTN: $GRTN^{PM}$+Raf^{Ci}. The two moieties become biochemically associated, and inactive Raf (Raf^{Ci}) is thereby affixed to the PM (Raf^{PMi}): $GRTN^{PM}$+Raf^{PMi}. This PM fixation process does not activate Raf. Next, the $GRTN^{PM}$ and Raf^{PMi} dissociate, either because RasGTP has been dephosphorylated or for other unspecified reasons, leaving us with $GRDN^{PM}$+Raf^{PMi} or $GRTN^{PM}$+Raf^{PMi}. The dissociated Raf^{PMi} is then activated, by means not yet elucidated: Raf^{PMa}. The latter has at least two fates. 1) Raf^{PMa} is simply deactivated and returns to the cytoplasm as Raf^{Ci}. 2) Raf^{PMa} phosphorylates MEK to initiate the MAPK cascade, and the inactive Raf returns to the cytoplasm as Raf^{Ci}.

While Raf^{PMi} is dissociated from $GRTN^{PM}$, the latter is still available for additional interactions with Raf^{Ci} or for other regulatory activities, including possible PM regulatory functions, as has been deduced from studies of Nfn functions in *Dictyostelium*.[1,2] Alternatively, when Raf^{PMi} dissociates from $GRDN^{PM}$, the latter is amenable to GEF conversion to $GRTN^{PM}$. Respecting the sequences considered here, it should be noted that interference with Raf^{PMa} production can derive from either deficient GAP function (e.g., mutant Nfn[4,5,8]) or deficient GEF function.[10] Deficient Nfn activity leads to excessively available GRTN and excessive Raf^{PMa}. Deficient GEF activity leads to inadequate generation of GRTN and insufficient amounts of Raf^{PMa}.

Ultimately, however, either of these two types of dysfunction leads to problems away from the PM: that is, Raf^{PMa} becomes Raf^{Ca}, and its interaction with MEK thus takes place in the cytosol. Under those circumstances, treating the over-production of Raf^{Ca} away from the PM has some logical validity. On the other hand, if the aberrant function of $GRTN^{PM}$ adversely affects the performance of the PM itself, treatment of cytosolic downstream elements ignores the PM disruptions and the functional PM problems that are likely to recur (or simply continue) when the cytosolic-focused treatment stops. This has been the experience to date: slowing or reversing neurofibroma growth during the treatment period, but recurrence of progression when the medication is stopped.

The key here is that production of Raf^{PMa} is NOT the sole function of the $GRTN^{PM}$ complex. In turn, treating (abrogating or compromising) the downstream consequences of Raf-based MEK activation still leaves a mutation-aberrant $GRTN^{PM}$ available to continue causing problems at the PM.

Thus, the primary concern is not on the indirect downstream adverse effects on the PM, but the direct and immediate influences of mutant Nfn on the definitive elements of the functioning PM, much as is seen in *Dictyostelium*.[1] Such immediate and direct adverse effects as an element of the PM might be manifest in aberrant chemotaxis, cell migration and phagocytosis. In addition, other membrane systems might be involved, including, for example, those involved in *melanosome* formation and placement. The intracellular and extracellular *macromelanosomes* that are part of NF1 *café-au-lait spots* (CLS)[11-13] may be a useful *in vitro* model for pursuing this line of reasoning. Moreover, it is tempting to consider that abnormal *NF1* SC PM functioning might account for the initial abnormal SC behaviors after disruption of the axon/SC relationship.

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