Asymmetry Pins Inscuteable

Deepa Subramanyam

During cell division, sibling cells of different developmental potentials can arise by the asymmetric localization of protein or RNA. A well-studied example is in the development of the fly peripheral nervous system, where the four cells of the sensory bristle arise from a single sensory organ precursor (SOP) through two rounds of asymmetric cell division. In the first round of division, the SOP divides to give a PIIa cell and a PIIb cell. Numb and Prospero (Pros) are localized in the PIIb cell which then divides to give a tertiary progenitor PIII and a glial cell. PIII expresses high levels of Elav and low levels of Pros and divides to produce the neuron and sheath cells. The glial cell expresses low Elav and high Pros and is recognized by the marker Repo. This cell migrates away from the other cells of the lineage following differentiation. The PIIa cell divides to give the hair and shaft cell. In loss-of-function numb mutants, the SOP divides symmetrically and gives two PIIa cells, which do not produce neurons. Asymmetric division is also used in the development of the central nervous system. Most Drosophila neuroblasts divide along the apical–basal axis to give a large apical daughter cell and a smaller basal ganglion mother cell (GMC). This asymmetry is due to the localization of proteins such as Prospero and Numb in the basal cortical region of the neuroblast (NB). The apical daughter cell retains the NB character and continues to divide as a stem cell. The basal GMC, on the other hand, can give rise to either neurons or glial cells (Figure 1a).

**Figure 1.** Division in (a) the peripheral nervous system; and (b) the central nervous system.

Insuteable localization to the apical cortex is responsible for creating asymmetry

The most important protein in the asymmetric division of NBs is Inscuteable (Insc), which has 859 amino acids and a SH3 domain. This protein is required for the localization of other proteins such as Pros and Miranda (Mir) which are responsible for asymmetric division. Drosophila NBs delaminate from a polarized epithelium in the ventral neuroectoderm and divide asymmetrically along their apical–basal axis. Ectodermal cells divide with the axis of their mitotic spindle parallel to the plane of the ectodermal layer. NBs divide with the axis of their
mitotic spindle perpendicular to the ectoderm\textsuperscript{5}. Insc is localized asymmetrically in the apical cortex of NBs during delamination as well as during the various stages of the cell cycle and becomes undetectable during anaphase. The localization of Insc to the apical pole of dividing neural precursors takes place before they enter prophase (Figure 2). Insc is also responsible for the correct orientation of the spindle axis in dividing delaminated NBs\textsuperscript{9}. As the cell cycle progresses, Insc-dependent apical localization of proteins such as Pros, Mir, Staufen (Stau) and Numb occurs before they are translocated to the basal pole.

**Insc maintains proper Pros localization**

Pros requires Insc for proper basal localization after a transient apical localization\textsuperscript{10}. Pros is a homeobox-containing transcriptional regulator which is required for proper neuronal differentiation, neuronal cell-fate specification, axon growth and guidance. Pros activates GMC-specific genes and represses genes which are normally expressed in NBs. pros RNA is found transiently localized in an apical crescent during late interphase and early prophase and is localized to the basal cortex during later prophase, metaphase, anaphase and telophase (Figure 2)\textsuperscript{9}. In insc mutants, Pros and pros RNA show apical localization and no basal crescent is detectable.

**Stau localizes pros RNA**

Stau, an RNA-binding protein has been identified in binding pros RNA. It was identified by using insc cDNA as a bait in a yeast two-hybrid screen. Stau is expressed in both GMCs and in NBs and is formed de novo in GMCs. This was proved by showing that Stau levels were very low in newly formed GMCs. Stau localizes to the apical cortex during interphase and early prophase and is then localized asymmetrical to the basal cortex during late prophase, metaphase, anaphase and telophase\textsuperscript{10}. The role of Stau in localizing pros RNA was discovered by the apical localization of pros RNA in stau mutants lacking both zygotic and maternal Stau contributions. Pros protein displayed normal basal localization in such mutants. Stau acts downstream of Insc because stau mutants display only pros RNA mislocalization, while insc mutants display defects in Stau. Pros and pros RNA localization as well as misorientation of mitotic spindles.

**Miranda – A multi-domain protein**

Mir, a multi-domain protein is required for the proper localization of Pros and Stau. It can also bind Insc. Mir too shows a transient apical localization during interphase and early prophase and then localizes to the basal cortex during the later stages of the cell cycle. Proper basal localization of Mir requires Insc. Loss-of-function mir mutation causes cytoplasmic distribution of Pros and its segregation into both GMCs and NBs. It is seen that localization patterns of Pros and Numb are disrupted by actin-depolymerizing agents like Latrunculin A and B, but not by treatment with a microtubule depolymerizing agent like colcemid. This shows that microfilaments play a role in the movement of proteins from the apical to the basal cortical regions\textsuperscript{15}.

**Bazooka maintains apical localization of Insc**

Bazooka (Baz), a cytoplasmic protein with 2 PDZ domains which has overall sequence similarity to Par-3 of *C. elegans* and rat ASIP has been shown to bind Insc and is responsible for its apical localization. In NBs, the spatial and temporal expression of Baz is similar to Insc. Both Baz and Insc localize to the apical cortex in the form of a crescent. In baz mutants lacking both maternal and zygotic contributions of Baz, apical crescents of Insc are not formed, and diffuse staining of Insc is seen. A fully penetrant spindle-orientation defect is also seen. baz mutant embryos also show pros RNA localized all over the cell cortex. Despite the lack of crescent formation, Pros was found localized in the GMCs. Hence Baz is required only for the early steps of Pros localization which lead to the formation of basal crescents during metaphase. In contrast, the segregation of Pros into the budding GMC during anaphase and telophase appears to be controlled by a Baz-independent mechanism. Baz localization, like Insc, is dependent upon the cell cycle and is visible as a
Pins interacts with Insc

Pins (Partner of Inscutable) is a novel interactor which is shown to be responsible for the maintenance of Insc and Baz apical localization. The initial localization of Insc and Baz is independent of Pins, but these three proteins depend on each other for the maintenance of their apical character. Pins was identified by two independent methods — a yeast two-hybrid screen, where the asymmetric localization domain of Insc (amino acids 288–497) was used as the bait to screen a 0–21 h embryonic cDNA library and immunoprecipitation coupled to mass spectrometry. Pins has 658 amino acids, and has a molecular weight of 71,523 Daltons. It has 7 TPR (tetra-tripeptide repeats) motifs at the N-terminal which are involved in general protein–protein interactions and is present in both epithelial cells and NBs. Pins also has three ‘GoLoco’ motifs which bind Goα and Gβi subunits and may represent a novel Goα-binding motif. Immunoprecipitation studies show that Insc and Pins interact under in vivo conditions. Pins binds in vitro to both Goα and Gβi (ref. 13). Using different segments of the Insc protein, it was deduced that the asymmetric localization domain of Insc interacts with Pins and that the N-terminal region of Pins is responsible for this interaction. Together these data suggest that Insc may act by recruiting Goα signalling proteins via Pins. As expected, it was seen that Pins localized to the apical crescent in NBs. Apical localization of Pins is seen only after delamination, whereas Insc forms an apical crescent during this process. This shows that Pins is not needed for the initial localization of Insc to the apical crescent, a process mediated by Baz. Pins crescents become more intense from prophase to anaphase of cell division, decrease during telophase and vanish after telophase. Insc and Pins are found colocalized after delamination.

In insc mutants, Pins is found distributed throughout the cortical region and is not asymmetric in distribution. Removal of zygotic and maternal Pins leads to the mislocalization of Insc and Baz in the embryo. Although the initial apical localization is normal, it cannot be maintained in the absence of Pins. baz mutants display abnormal localization of Pins and Insc. It is observed that pins loss-of-function displays a similar phenotype as insc loss-of-function mutation. It is characterized by the defective orientation of the mitotic spindle, by mislocalization of crescents of Mir, Pros, Numb and Partner of Numb (Pon). The phenotype differs slightly from that of insc loss-of-function in that the crescents showed a greater frequency of overlap with one of the poles. This shows that the coordination of mitotic spindle orientation with protein localization may be less disrupted in this mutant than in the loss-of-function of insc. Resolution of the distinct fates of the neurons RP2 and RP2sib formed from GMCs also frequently fails to occur. Often, duplicated RP2 neurons are formed at the expense of RP2sib.

Implications of the Pins discovery

The pins loss-of-function phenotype shows that the initial localization of Insc to the apical crescent needs Baz and is independent of Pins. At later stages of the cell cycle, this mutant causes mislocalization of Baz, Insc, Stau, Pros, Numb and Pon, showing that Pins is needed for the maintenance of the localization of these proteins as opposed to their initial localization. Although no direct interaction has been shown between Pins and Baz, we know that Baz interacts with Insc which in turn interacts with Pins. The maintenance of the localization of Baz, Insc and Pins during later stages of the cell cycle is interdependent. The maintenance of apical-basal asymmetry may occur through the G-protein signalling cascade.

How Pins is necessary for the maintenance of Baz and Insc apical localizations is a mystery that remains to be solved. The interdependence of all the three interactors could suggest a mechanism involving some sort of complex necessary for the microfilament-dependent localization of the basal crescent elements. How these three apical interactors direct basal migration is a field open to speculation. Do G proteins play a role in the translocation of basal-crescent elements? If so, what is the downstream signalling pathway involved in this process? These are questions which still continue to interest researchers in the field of asymmetric cell division.
Global ice cover – A Neoproterozoic puzzle

A. V. Sankaran

During the Mesoproterozoic, i.e. 1600–900 million years (m.y.) ago, several ancient continental crusts accreted to form the supercontinent Rodinia\(^1\). Subsequent fragmentation of this supercontinent 750 m.y. ago, leading to the creation of new oceans, and their collision again around 600 m.y. ago, resulting in global mountain building (Pan-African orogeny) are some of the well-known Proterozoic (2500–540 m.y.) events\(^3\). Lesser known episodes of this period are the series of climatic ups and downs causing massive glacial and interglacial spells, development of oxygen in the atmosphere, banded iron formations (BIF) and the evolution of eukaryotes (Figure 1 \(a\)). At least four ice ages, perhaps five\(^4\), of which the earliest one ~ 2.3–2.4 billion years (b.y.) ago and the last during the Neoproterozoic (~ 900–540 m.y.) have all evoked considerable interest\(^5\)–\(^8\). Especially the last spell of ice age during the Neoproterozoic, lasting for 10 m.y., hundred times longer than the later period Pleistocene (1.8–0.1 m.y.) glaciation, was thought to have completely covered the earth under a thick blanket of ice, creating a veritable ‘snowball earth’\(^9\).

The possibility of a great ice age occurring during Neoproterozoic was advanced more than three decades ago after the discovery of glacial deposits, interspersed with sedimentary rocks in several continents which were then located in the low latitudes of the earth\(^6\). Geologists did not accept this view of a global ice age and it had, therefore, remained dormant for many years till its recent resurrection after new findings on limestones capping Neoproterozoic glaciogenic rocks in Namibia testifying to glacial and interglacial climatic recurrences\(^5\),\(^10\),\(^11\). India too was part of the Mesoproterozoic continental assembly and though it has exten-

---

**Figure 1.** a, Precambrian time-scale showing major geological events during the Proterozoic period; b, Break-up of supercontinent Rodinia and distribution of lands over low latitude equatorial zone ~ 750 m.y. ago; c, Earth’s geographic zones.
sive sedimentary rocks spanning the entire Proterozoic, the few glaciogenic tillites, diamicittes and boulder beds reported from this period proved to be controversial. These rocks which occur in Sone and Ken Valleys (Madhya Pradesh), within the Lower Vindhyan formations, are assigned Meso to Palaeoproterozoic in age according to geochronological data but considered much younger (late Neoproterozoic to early Cambrian) according to biostratigraphic correlation. Much discussion had centered around a ~ 50 m thick tillite bed occurring here – the Gangau tilloid, having glaciogenic features like assortment of angular and subangular clasts of varying sizes and lithologies, including a few granite boulders (from the basement granite), some of them distinctly faceted and striated. This tillite bed is considered as the only known Precambrian glaciogenic deposit in India. A bed of limestone, intervened by a layer of sandstone, succeeds the Gangau tillite and hence the transition here is not abrupt, as noticed in the other glacial bed successions elsewhere in the world. Yet, this succession is noteworthy in the light of palaeoclimatic inferences about glaciation which the discovery of carbonates capping glaciogenic deposits in Namibia had recently provided. But palaeomagnetic, sedimentological and geochemical studies of this Gangau tillite bed have currently rejected the glacier connection and have, instead, described them as continental debris-flow generated from ferruginous regolith and deposited over 44.7° mid-latitudes.

Though undoubted evidences for the Neoproterozoic glaciation are available, their spread over the entire earth has raised two major issues. Firstly, palaeomagnetic reconstructions have indicated clustering of landmasses of this period over the equator (Figure 1 b), a region too warm to support an ice cover. Secondly, skeptics feared that once under complete ice cover, the earth could never be retrieved since white ice with its high reflectivity would reflect back more and more solar radiation, thus aiding further cooling. Such an ice-covered earth, they argued, will end up as a dead planet with all marine life wiped out. However, this extreme view did not prevail long as several multidisciplinary researches carried out during the last ten years could explain not only how deep freeze conditions over tropical equatorial zone are achievable, but also how the planet could burst back into life from such an entombed state. In fact, in the opinion of a section of scientists, except for some of the photosynthesizing organisms that require sunlight, there were plenty of other microbial forms whose energy needs were met from abiotic sources and a combination of oxidants and reducers. These sources were available through ocean bottom hydrothermal vents, niches in continental ice cover over lands permitting not only sunlight but also entry of oxygen; and for some of the primitive organisms, through methanogenesis. Also recent computer simulation studies incorporating ambient parameters of the period and present-day CO2 levels in the atmosphere revealed evolution of rapid glacial and interglacial conditions in the equatorial region. More importantly, the climate–ice-sheet interaction model showed development of a belt of open water refugium for protecting life and enabling its diversification.

Climatic physicists, through simple energy-balance equations modelled catastrophic freezing over the entire earth surface under favourable conditions likely during Proterozoic and earlier times. They showed how a small reduction in solar luminosity could progressively develop ice and snow cover towards the equator and how this cover would reflect more solar radiation back into space (surface albedo) to further cool and extend the ice cover over the entire planet. The Precambrian sun was 7% dimmer than the present-day sun and under increased surface albedo, which is more from low latitude regions where surface area per latitude is greater, conditions were ripe for a runaway deep freeze. Under this ice-albedo feedback, even the oceans can develop a thick cover of ice, except at the bottom where heat flow from the earth’s interior will prevent freezing. Further, the decrease in greenhouse warming due to depletion of CO2 through uptake by the marine life evolved abundantly in the wake of...
opening up of new oceans (Pacific and Iaepetus) can strengthen this phenomenon. A few scientists, however, have questioned the ice-albedo model; in their view reduced luminosity could not be the cause because the luminosity of the early sun is believed to have been still lower before the break-up of Rodinia. According to their recent calculations, reduction in sun’s luminosity should be > 10% to bring about an ice cover over the equator. The susceptibility of an early earth to irreversible ice cover, even under low solar fluxes of 25 to 30%, is discounted because of the formation of highly reflective CO₂ clouds accumulating from early volcanoes having higher outgassing potential, thereby creating a negative ice-albedo feedback to keep the planet warm and prevent catastrophic deep freeze.

Besides the ice-albedo feedback process, an alternative hypothesis advanced attributes to the Precambrian earth’s peculiar orientation with respect to the sun for the development of ice cover over low latitudes. According to this view, the earth’s obliquity (tilt or angle between the earth’s spin and orbital axes) was greater than 54° during most of the earth’s early history. At this orientation, the equator received less solar radiation and was the coldest place on earth covered by ice (Figure 2). But the view does not support a global ice cover nor mention how the earth acquired the present-day tilt of 23.5°. This decline to present obliquity, which it acquired by Midpalaeozoic (430 m.y. ago), is now explained by obliquity–oblateness feedback theory. According to this theory, the gravitational forces exerted by the sun and the moon slowly affect the geometric parameters of the earth’s orientation in its path around the sun. This makes both its spin axis and orbit plane to precess, i.e. wobble, causing the obliquity to oscillate ±1° over a period of 41 ky. The spin precession rate also gets affected when the earth’s mass distribution (for example, lopsided distribution of ice cover or lands) is not balanced. Over geologic periods, the latter precession tends to decrease the obliquity, leading to gradual warming up of the equator and cooling of the poles (Figure 2). Another explanation for the rapid decline from the high to low 23.5° tilt of the earth invokes the true polar wander (TPW) event, which commenced during the late Precambrian/early Cambrian period. This TPW was caused by an imbalance in the mass distribution of the earth, which is forced by the laws of physics to adjust to a more stable configuration in comparatively rapid time-scales through movement of the lithosphere and mantle. Called the inertial interchange TPW event, this resulted in the earth’s spin axis moving closer to its orbit axis, which, however, had remained stable around the sun. The high obliquity view for low latitude glaciation has also been disputed by a few who point to the presence of typical freeze-thaw polygonal structures in frozen soil (permafrost regolith) in South Australia, indicative of seasonal variations of temperature, unlikely if ice indeed had blanketed the entire equator.

Once the earth came under runaway glaciation, a reversal to warm cycle through accumulation of CO₂ in the atmosphere was inevitable since the normal balance maintained between the atmosphere–ocean system ceases during ice cover. Steady build-up of CO₂ in the atmosphere takes place through volcanic releases. Absence of CO₂-consuming organisms and silicate weathering reactions to produce carbonates augment this build-up. At a critical concentration of nearly 120,000 ppm of CO₂, which is 300 times the present-day level, transformation of ice-house to greenhouse conditions takes place aided by warm land temperature (up to 50°C). Melt down of ice cover follows, which reactivates the atmosphere–ocean exchange and liberates water vapour bringing copious carbonic acid-rich rain. Chemical weathering of land surface ensues, producing abundant carbonate-rich run-off to be deposited as limestone cap over the glacial debris already downloaded when the glaciers thawed. These post-melt-down processes take place in a very short geological time which thus explains the abrupt transition from glacial sediments to carbonate cap observed in several localities.

The postulated climatic and biotic swings of the Neoproterozoic period and accompanying bouts of extinction and explosion of life are reflected in the shifts in the proportions of the carbon isotopes (¹²C and ¹³C) in the carbonates formed prior to and following glaciation. Their normal proportion, as in volcano outgassed CO₂, is about 1% ¹³C and 99% ¹²C. Marine organisms like the photosynthesizing algae and microbial colonies prefer ¹²C to the heavier ¹³C when they convert CO₂ to sugar. Hence, ¹³C enrichment is seen relative to volcanic source in marine carbonates when such life forms are abundant in oceans and a swing back to normal ratios occurs when these life forms decline. Such variations, which reflect prevailing biota as well as climate, have now been used in the interpretation of Neoproterozoic deep freeze.

About 800 carbonate samples of this period, lying below and above glaciogenic diamictite beds in Namibia (Congo craton), were analysed for these isotopes. These carbonates formed on a slowly subsiding continental shelf between 12° S and 39° S latitude, provide an unbroken carbonate sequence, preserving a good record of Neoproterozoic carbon cycle. In these rocks formed prior to glacial ice cover, ¹³C was in excess, by as much as 1.5% relative to volcanic CO₂. This indicates ¹²C uptake from ocean waters by the flourishing organisms. But shortly before the onset of ice age, the ratio, as prevailing in the carbonates closer to the glacial bed, is observed to swing back to normal volcanic proportions reflecting decline or extinction of the biota. This normal ratio persisted through several metres thickness of cap carbonates lying over the glaciogenic beds before slowly springing back to higher levels of ¹³C, with gradual proliferation of marine life following the onset of post-ice age warming period.

The nature of iron and uranium deposits during Palaeo and Neoproterozoic periods also provides clues about global ice cover through shifts in ocean chemistry. These are elements sensitive to oxygen fluctuations that occur in the oceans while under anoxic and oxic phases developing during glacial and interglacial periods, respectively. Most of the banded iron ore deposits (BIF) of the world are associated with strata older than 1800 m.y. (Indian occurrences in eastern and southern parts are > 3 b.y. old), formed at a time when the atmosphere had input of free oxygen arising from increased burial of organic matter. Besides, essentially as oxides, the sulphuric iron deposits also occur during this period. The abundant iron that was present as soluble ferrous (Fe²⁺) iron got precipitated as insoluble ferric iron (Fe³⁺), in the BIF, when the deep anoxic ocean waters were brought by upwelling currents to near surface zones richer in oxygen or
through downwelling of oxygen-rich currents\textsuperscript{37}. Another route forming the iron deposits is the reduction of ferrous iron present as sulphate to insoluble sulphides during ocean anoxia phase, a reaction predominant in Palaeoproterozoic times\textsuperscript{38}. Precambrian stratigraphy, thereafter, shows a hiatus in BIF occurrences for nearly a billion years after which they appear again. These swings are now explained as resulting from anoxic conditions in the oceans under ice cover (hiatus period, when iron is held in the waters as soluble Fe\textsuperscript{2+} or reduced as sulphides) which return to oxic condition (when oxidation of Fe\textsuperscript{2+} to Fe\textsuperscript{3+} resumes) after the melt-down of ice\textsuperscript{37}.

Critics\textsuperscript{28,35,39} have doubted the global ice cover hypothesis and development of low latitude glaciation and have expressed that the high thermal capacity of oceans and wind-driven thermohaline currents will prevent freezing of the oceans. Also, the high obliquity-earth postulate to explain low latitude glaciation appeared questionable in the absence of evidences for the conceived warm pole and cold equator. The anomalous presence of carbonate sediments in some localities interspersed with glacial beds and absence of any sea-level rise that should have accompanied melting of ice of global magnitude are other issues, the critics point out, that go against a snowball earth view. However, proponents have explained through computer simulations and other studies how some of these field anomalies can occur and have defended their views\textsuperscript{30,40–42}. More importantly, they have emphasized how the snowball hypothesis simultaneously explains many of the issues like catastrophic glaciation over low latitudes and the duration, the general occurrence of cap carbonates over glaciogenic beds in a number of countries, the observed sharp transitions between the two beds, the C-isotope excursions, as well as the much debated Cambrian explosion\textsuperscript{43} of life triggered by extreme conditions of stress that prevailed at the end of the Neoproterozoic ice age\textsuperscript{44,43,44}. Today, the low latitude glaciation of this period has been recognized. But, as aptly remarked by some, the snowball hypothesis will have to wait for recognition by earth scientists if one goes by their customary response to some of the earlier ideas like continental drift, plate tectonics, extra-terrestrial impact-related extinction of life and the dinosaur–bird link. Whether the earth during the Neoproterozoic was a snowball or not, the postulate has triggered several fascinating researches in its wake and earth science has greatly benefited.

16. Mahadevan, T. M. (pers. commun.).

A. V. Sankaran lives at 10, P & T Colony, I Cross, Jl Block, R.T. Nagar, Bangalore 560 032, India.