

## Compensasome in *Drosophila*

Ribonucleoprotein (RNP) complexes such as ribosomes, spliceosomes, primosomes and telomerases play important roles in different cellular regulatory activities. Recently, a novel RNP complex has been reported in *Drosophila*. The complex concerns dosage compensation, wherein it promotes the enhancement of transcription of single male X chromosome, so that the quantum of products produced by the single X chromosome in a male is equal to that of the two X chromosomes of females. Aptly, this ribonucleoprotein complex has been named the “compensasome” (Franke and Baker 1999).

The known protein components of the compensasome include the products of five male specific lethal genes (*msls*). They are *msl1*, *msl2*, *msl3*, maleless *mle* and males absent on the first – *mof*. MSL1 is a novel acidic protein, MSL2 a putative zinc binding RING protein, MSL3 a chromo domain protein, MLE a DNA/RNA helicase of the DEAH subfamily and MOF is a protein with an acetyltransferase domain (reviewed by Lucchesi 1998). The non-coding RNA component of the compensasome is coded by two genes, *rox1* and *rox2* (Amrein and Axel 1997).

Two successive steps have been visualized for the recognition of the X chromosome by these components of the machinery. The MSL1 and MSL2 interactions initiate the formation of the complex. They are found to associate with 30–40 sites present all along the X chromosome; subsequently, they recruit other MSL proteins to these sites. Of these sites on the X chromosome, two encode *rox* RNAs, and the *rox* RNAs are incorporated into the growing MSL complex. In the next step, the MSL–RNA complex associates with chromatin entry sites specifically on the X chromosome and spreads to other sections of the X chromosome (Kelley *et al* 1999). Elegant immunofluorescent studies involving *rox* antisense RNA and MSL antibodies have shown colocalization of these on the single X chromosome of males and their absence on the double X chromosomes of females (Franke and Baker 1999).

The MSLs appear to function through chromatin remodelling of the X chromosome. Under the influence of the MOF protein, histone H4-Lys 16 acetylation occurs. Histone acetylation and gene transcription are related. Many proteins designated as transcriptional cofactors possess histone acetyl transferase activity (Brownell *et al* 1996). With regard to role of *rox* RNAs, the following possibilities are being tested. They may act as contact point between MSLs and the X chromosome, or its interaction with RNA polymerase or other components of the chromatin remodelling machinery to enhance transcription within a stipulated time.

In contrast to the coordinate upregulation of the single male X chromosome in *Drosophila*, the equalization of X-linked products between the sexes (dosage compensation) takes place by two other (and different) mechanisms in humans and nematodes (*Caenorhabditis elegans*). In humans, it is through inactivation of one of the two X chromosomes in females while in the nematode it is achieved through hypoactivation of both the X chromosomes in females (reviewed by Lucchesi 1998; Lyon 1999). With the conceptualization of compensasome in *Drosophila*, attempts have been made to analyse the nature of compensasomes in an inactivated (humans) and hypoactivated (nematode) dosage compensation system. Preliminary reports show parallels between *rox* RNAs of *Drosophila* and *xist* RNAs of man.

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## Size control in development: lessons from *Drosophila*

As long back as in 1927 J B S Haldane pointed out that “The most obvious differences between different animals are differences in size, but for some reason the zoologists have paid singularly little attention to them” (Haldane 1927). Almost three quarters of a century later, one of the most important and fundamental aspects of development – how the size of a multicellular organism is determined – remains as mysterious as ever. The final size reached by an adult organism is a consequence of changes in the size and number of cells during its development. The critical factors thus are how many cells there are, how big a cell is on average and the amount of extracellular matrix and fluid present.

Early studies on the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* provided indications that growth could continue in the absence of cell division. Therefore growth was not simply a matter of increasing cell numbers; control of cell size was important too. Unfortunately, while much attention has directed to the analysis of mechanisms which regulate cell numbers, cell size regulation seems to have received a less than fair treatment by researchers. However, recent studies on the fruitfly *Drosophila melanogaster* have shown that mutations that block cell cycle progression tend not to arrest cell growth (Conlon and Raff 1999). Therefore the mechanisms that regulate the sizes of the imaginal discs (sacks of epithelial cells which reorganize during metamorphosis into external body parts such as the antennae, wings and legs) during larval development must be acting primarily by regulating cell size and not by regulating cell numbers. How then is size regulated? We know that growth depends on intrinsic cues as well as extrinsic factors which can stimulate intracellular pathways and induce biosynthetic processes. In vertebrates the insulin-like growth factor IGF-1 is one such extrinsic factor that regulates cell growth and proliferation. Molecules involved in the insulin signalling pathway in vertebrates – for example, insulin receptor, insulin receptor substrate and phosphatidylinositol 3-kinase – also regulate cellular growth rate and/or cell size in *Drosophila* (Leervers 1999).

In a recent report Montagne *et al* (1999) identify *Drosophila* S6 kinase (*dS6K*) as a signalling molecule which when mutated, slows growth and reduces cell size and thereby body size. In order to analyse the function of *dS6K*, Montagne and colleagues isolated flies with null mutations in the *dS6K* gene. They found that the mutant flies were delayed in development and were severely reduced in body size (around 60% reduction in body weight and 30% reduction in cell size in homozygous female flies). However, all the body parts were affected to the same extent, implying that proportions were preserved. The latter observation is in accord with recent results showing that the overall form of an organism can be influenced by a competition for developmental resources between different body parts (Nijhout and Emlen 1998). After examining the wings and eyes Montagne *et al* (1999) arrived at the conclusion that the mini-flies were made up of cells which were reduced in size. The total number of cells in the body remained more or less the same. The smaller cell size could have come about because the cells were dividing earlier than usual or because the flies were emerging from developmental delay without complet-

ing the last round of cell growth. To examine these possibilities the authors analysed the imaginal discs of the mutants. It turned out that the discs were smaller than usual (as expected) and that the cells in them, which were also small, grew at a slower rate than normal. Thus, in the absence of *dS6K*, a miniature but perfectly formed fly containing the normal number of cells is formed.

In vertebrates the S6 kinases regulate the synthesis of a family of proteins, primarily components of the translational apparatus, in response to the insulin signalling pathway. In S6K1 deficient mice, the derived liver and embryonic cells show unimpaired phosphorylation of S6 thanks to compensation by S6K2 but the gene disruption results in a small mouse. The involvement of protein synthesis in growth control in *Drosophila* is not surprising, a class of mutants called *Minutes* are known to delay development and slow growth rate and division. Also, recently a screen to identify genes required for larval growth identified a translation initiation factor, Eif4A. *Minute* genes encode components of the ribosomal translational machinery. However, these mutations do not alter cell size, showing that cell growth and cell division are normally coupled in order to maintain an appropriate cell size.

This suggests that in the *dS6K* mutant, growth *per se* is not severely impaired; the number of cell divisions required to maintain normal cell numbers still occurs. Thus, *dS6K* identifies a branch of the insulin signalling pathway likely to be involved in growth control by affecting growth at the cellular level and thereby modulating organ and body size. The challenge now is to find out how this signalling network is controlled in response to environmental and developmental cues. We need to understand what switches it on, and most importantly what switches it off, as this may be the way in which the final body size is determined.

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## Should erythrocyte destruction *in vivo* be through phagocytosis alone?

The human body has  $3$  to  $5 \times 10^{13}$  erythrocytes in circulation. It is estimated that the average life span of an erythrocyte is about 100 days (50 days in rodents) (Bocci 1981; Landaw 1988). About 1% of all erythrocytes (2% in rodents) must therefore be killed each day and in order to sustain the levels of circulating erythrocytes, an equal number must be added daily to the circulation. The processes of erythropoiesis and erythrocyte destruction must be finely balanced to ensure that the required number of erythrocytes stay in circulation, and must be flexible enough to meet contingencies arising due to a sudden loss of blood or an increased demand in oxygen carrying capacity in hypoxic conditions. Erythrocyte numbers can change and remain high (polycythemia) or low (anemia), and this must be

accounted for by altered rates of erythrocyte generation and/or destruction. In order to understand the homeostasis of erythrocytes, the mechanisms involved in their generation and destruction must be clearly understood. While a great deal is known about erythropoiesis and the role played by a variety of cytokines and growth factors in this process (Barosi 1994; Miller *et al* 1994; Jacobsen 1995; Roeder *et al* 1998), the mechanisms involved in destruction of erythrocytes are not well understood. It is believed that aging erythrocytes are trapped in the spleen and bone marrow where phagocytic cells of the reticuloendothelial system remove them (Galili *et al* 1986; Connor *et al* 1994). How aging erythrocytes are recognized by the phagocytes is not clear, though there are several speculations in the literature about the process of senescence of erythrocytes and the changes that may lead to their recognition by the reticuloendothelial system (Hensley *et al* 1989; Kay *et al* 1989; Kosower 1993).

In general, there are two basic mechanisms of removal of unwanted cells in biological systems. These are (i) apoptotic changes or other discernable changes like opsonization of damaged cells, leading to their recognition and phagocytosis by scavenger cells like macrophages, and (ii) direct lysis of cells which can be mediated either by cellular effector elements like cytotoxic T cells and NK cells, or through the activation of the complement system. If cells are killed by a lytic process, internal organelles and debris released by the lysed cells may induce an inflammatory response which may be injurious to the surrounding tissue. On the other hand, if the damaged target cells are phagocytosed, inflammatory responses may be avoided. It should be noted that erythrocytes lack any internal organelles and as such the danger of induction of an inflammatory response following their lysis should be minimal. So far however, the literature lacks specific information about the participation of the lytic pathway in erythrocyte destruction. If phagocytosis is the sole mechanism of erythrocyte destruction, an estimated 2–10 billion circulating monocytes/macrophages along with additional phagocytic cells of the reticuloendothelial system may be involved in phagocytosis of 300–500 billion aged erythrocytes each day. The sheer magnitude of the job of destroying such large numbers of aged erythrocytes makes one wonder why nature would confine all erythrocyte destruction activity to phagocytes and desist from using the lytic pathway to kill aged erythrocytes? It is possible that erythrocyte lysis does take place but has escaped notice since its demonstration is relatively difficult. If erythrocytes are co-cultured with immunocytes, there would be morphological evidence for phagocytosed erythrocytes in the form of internalized erythrocytes. Lysed erythrocytes on the other hand would simply vanish from the culture leaving no trace. Lack of published evidence for lysis of erythrocytes could thus be due to the fact that phagocytosis is easily discernable whereas lysis is not.

We have explored this issue recently and have presented evidence indicating that murine erythrocytes are lysed efficiently by immunocytes *in vitro* (Saxena and Chandrasekhar 2000). We observed that the number of erythrocytes decline progressively if cultured in the presence of leukocyte preparations derived from murine spleen, bone marrow and thymus. The decline was not entirely due to phagocytic activity because depletion of phagocytic cells did not abolish the erythrocyte depleting activity (EDA) of leukocyte preparations. In addition, fixing of effector leukocyte preparations by paraformaldehyde treatment did not lower their EDA, strongly suggesting the involvement of non-phagocytic mechanisms in erythrocyte destruction. Assessment of erythrocyte lysis by the chromium release assay of cytotoxicity also provided direct evidence of erythrocyte lysis by leukocytes. T cells appear to be indirectly involved in EDA associated with leukocyte preparations because spleen cells from athymic nude mice were devoid of erythrolytic activity and EDA of normal mouse spleen cells was boosted by the T cell mitogen Con-A. Finally, we provided evidence for the participation of Fas–FasL and TNF–TNFR interactions in cell mediated lysis of erythrocytes. These results indicating that besides phagocytosis, direct lysis of erythrocytes is possible, may have far-reaching implications. Since the homeostasis of erythrocytes in blood circulation is crucial for sustaining life, factors which participate in erythrocyte homeostasis should be clearly defined and their regulation understood. A novel lytic pathway for erythrocyte destruction should focus attention on gaining understanding of the extent to which this pathway contributes to actual destruction of erythrocytes *in vivo*, and the factors which regulate this pathway of erythrocyte destruction. We have no information at present regarding whether, and to what extent, direct lysis of erythrocytes contributes to the turnover of erythrocytes *in vivo*. While there are methods available to study the overall turnover of erythrocytes *in vivo*, appropriate experimental approaches need to be developed to estimate the relative importance of different

possible pathways in the whole animal. We are at present trying to adapt the chromium release assay of cytotoxicity for this purpose.

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## Transfer of learning across the somatosensory cortex

It has long been known that the somatosensory cortex in the human brain contains a ‘map’ of the human body. The map is made up of columns of cortical tissue within which the cells respond best to stimulation of ‘their’ designated body part. Such body maps have been seen in virtually all animals that have been studied. The maps have been shown in some cases to be dynamic, in the sense that experience can alter their precise structure. It appears that the brain at birth is given a particular map, which it then adjusts according to what it experiences in its lifetime.

If this is true one can ask, Do neighbouring regions of the cortex share their information? To address this question, Justin Harris at the University of New South Wales, Australia and Mathew Diamond and Rasmus Petersen at the International School of Advanced Studies, Trieste, Italy, decided to utilize the well-studied rat whisker system. The rat’s whiskers are arranged on the side of its snout in a neat five by seven matrix. Correspondingly there is five by seven matrix of cortical tissue columns in the somatosensory region of the rat’s cortex.

The rats were firstly trained in the Gap Cross Task. This task required rats placed in the dark on a plat-

form to detect the edge of a second platform using only a single whisker (all the rest were clipped off). Once the rats had mastered this task – or, in a manner of speaking, once the whisker had mastered this task – the single whisker was clipped off, and it was glued onto the stub of some other whisker. Now the rats were immediately tested for their ability to perform the Gap Cross Task. Rats that had their ‘prosthetic’ whisker on the stub of the original (trained) whisker, could perform the task as well as before. Rats with the prosthetic whisker on a neighbouring stub needed a few trials to perform the task well. Rats with prosthetics on whisker stubs further away could not perform well at all, and they took as many trials to re-learn the task as did naïve rats. Interestingly, when the ‘prosthetic’ whisker was on the stub corresponding to the trained whisker, but on the *opposite* side of the snout, the rats could once again perform the task very well.

The next step was to develop a simple test to look at such a transfer of learning in humans. Blindfolded human subjects were taught to discriminate a smooth and a rough surface by using just one finger. Subsequently, still blindfolded, they were presented with either of the discriminanda at the tip of the trained finger, and asked to judge if it was the rougher or the smoother one. The task was said to have been learnt when the subjects got ten correct answers in a row. To examine the transfer of this learning, the same task was repeated, except that the subject now had to use a different finger. It turned out that, as in the case of the rats’ whiskers, nearby fingers performed quite well; the subjects made fewer errors with fingers adjacent to the trained finger. Even more strikingly, the trained finger’s equivalent on the opposite hand did as well as a finger adjacent to the trained finger in discriminating the surfaces correctly.

This is a remarkable example of a principle of cortical organization that is shared between species well separated by evolutionary time. Work in progress involves an attempt to use electrodes implanted in the whisker-cortex of rats in order to monitor in real-time the dynamical changes that accompany experience-dependent plasticity in the cortex.

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