Islet neogenesis from adipose tissue derived stem cells
FROM THE SECRETARIAT

Dear Members

This is the second issue of ISCB Newsletter (Volume 28, Number 1) being brought out by our team. Due to the encouraging response to the preceding issue of the newsletter, we have endeavored to make the current issue even more interesting. We hope that you will enjoy the excellent articles on various aspects of cell biology, written by Drs Robin Mukhopadhyaya, V. Nagaraja and Surajit Sarkar. A report on the proceedings of the XXXI All India Cell Biology Conference and Symposium on Symposium on Stem Cells: Application and Prospects held at Varanasi in December 2007, prepared by Dr. J.K. Roy, has been included for the benefit of those who could not attend the meeting. An additional attractive feature of this issue is a crossword puzzle designed by Tanvi Sinha and Shahnaz Masani, second year M.Sc. Zoology students at University of Pune. We urge all members, young and not so young, to send in their entries by 31st August 2008.

The XXXII All India Cell Biology Conference will be held at Agharkar Research Institute, Pune between 4th and 6th December 2008. This year’s meeting will include a symposium on Stem Cells and Pattern Formation. Although we had a symposium on stem cells last year, the emphasis this year will be different. Stem cell biology is too vast an area to be covered in one symposium. We urge members and potential members of ISCB to participate in the meeting.

We have begun to update the ISCB website (http://www.iscb.org.in) and request members to visit the same.

Vidya Patwardhan Bimalendu B. Nath Surendra Ghaskadbi
Treasurer Joint Secretary Secretary

On the cover: Islet neogenesis from adipose tissue derived stem cells. (Contributed by Dr. Ramesh R. Bhonde, National Centre for Cell Science, Ganeshkhind, Pune-411 007)
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Cytogenetics Laboratory, Department of Zoology, Banaras Hindu University, Varanasi 221 005 Telephone : +91-542-2368145; FAX : +91-542-2368457; E-mail : jkroy@bhu.ac.in (Dr J K Roy, Executive Secretary for a period of five years from April 1, 2004 to March 31, 2009)
Conference announcement and First Circular

XXXII All India Cell Biology Conference
&
Symposium on “Stem Cells and Pattern Formation”

December 4-6, 2008
Agharkar Research Institute
G.G. Agarkar Road
Pune-411 004

Contact e-mail: 32aicbc@gmail.com

It gives us great pleasure in inviting you to the XXXII All India Cell Biology Conference, the Annual Meeting of the Indian Society of Cell Biology. The conference has been planned for 3 days and will cover various aspects of cell biology through Invited talks, Prof S P Ray-Chaudhuri 75th Birthday Endowment Lecture, Proffered Talks and Posters. The meeting will include a Symposium on ‘Stem Cells and Pattern Formation’.

Confirmed speakers: Sudha Bhattacharya (New Delhi), Amitabha Chattopadhyay (Hyderabad), Jyotsna Dhasaw (Hyderabad), S.E. Hasnain (Hyderabad)*, Durgadas Kasbekar (Hyderabad), S. Mahadevan (Bangalore), Rita Mulherkar (Navi Mumbai), Veena Parnaik (Hyderabad), Rajiva Raman (Varanasi), P.B. Seshagiri (Bangalore), Imran Siddiqi (Hyderabad), David Strutt (Sheffield), Gerhardt Technau (Mainz), Surekha Zingde (Navi Mumbai). *Final confirmation awaited

Stem cells and pattern formation: Makoto Asashima (Tokyo), Thomas Bosch (Kiel), John Gurdon (Cambridge)

All those interested in participating in the meeting may inform us by email latest by 31st May 2008 on the address given above (32aicbc@gmail.com). The second circular will be sent to them by email. Please visit ISCB website http://www.iscb.org.in

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Transcription initiation – multistep activation as a mechanism to activate gene expression

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Regulation of gene expression

Regulated expression of genes is central to growth and development of an organism. Consequently, a varied repertoire of mechanisms for the control of gene expression has evolved. The precise timing and frequency of expression of genes in response to environmental stimuli and biological requirements is controlled at the level of transcription and/or translation. Transcription, which is the first step of expression, proceeds in three distinct stages: initiation, elongation and termination. Regulation operates primarily at the level of transcription initiation, brought about by sequence-specific DNA binding proteins. A single, multi-subunit RNA polymerase (RNAP) is involved in transcription in prokaryotes. The enzyme is composed of two α subunits, a β and β’ subunit, omega and one of the sigma subunits, which confer promoter specificity. In Escherichia coli, $\sigma^{70}$ is the major sigma subunit. The other sigma factors in the cell include the $\sigma^{32}$, $\sigma^{54}$, $\sigma^{28}$, $\sigma^{E}$ and $\sigma^{s}$, which have different promoter specificities. Regulation of gene expression is also exerted at some other steps. Post-transcriptional control at the level of mRNA stability, post-translational modifications, protein turnover and stability are some of the other ways to optimize the gene expression. Control at the initiation of transcription is most favored since it is cost effective for the cell, obviating the need for expending energy at later steps viz. transcription elongation, termination and translation. The basic steps of promoter-polymerase interaction needed for initiation of transcription is shown in Fig.1.

Classical paradigms of transcriptional control in bacteria are simple yet elegant in their execution. In the classical model, negative control is exerted by repressors or negative regulatory proteins which prevent transcription initiation at the promoters. The repressor inactivation or dissociation from its operator site ensures transcription initiation by RNA polymerase. In contrast, in positive control mechanism, the polymerase requires the action of an activator or positive regulatory factor for transcription initiation. In genes or operons subjected to positive control, normally, the promoters are not “strong” comprising of sequences away from consensus -10 (TATAAT) and –35 (TTGACA) elements. Typically in such weak promoters, the interaction of the polymerase is less efficient and in some cases highly compromised. The activators assist polymerase in more ways than one in ensuring efficient initiation of transcription process. They influence different steps described in Fig.1.

Transcription factors, by virtue of their exquisite specificity to target DNA and interaction with RNA polymerase, direct transactivation from a variety of promoters. Thus, positive control is effected in several different ways. The activators modulate the structure of DNA by bending or looping mechanisms. The net result is the contact achieved between the activator and the polymerase to influence the transcription initiation process. A large body of literature has accumulated on transcription activators from different prokaryotic organisms. Attempts have been made to identify the specific interaction of the transactivator protein with RNA polymerase by genetic and biochemical techniques. The positive control mutants of global regulator CRP and bacteriophage $\lambda$ CI have been isolated which have normal DNA binding properties but are unable to participate in transactivation. Such mutants of CI repress bacteriophage $\lambda$ strong rightward promoter $P_R$ in normal fashion but have minimal role in activating its own transcription at $P_{PRM}$. Compensatory mutants of RNA polymerase have
been isolated in which transactivation is restored and these mutations map to the alpha or sigma subunit of RNA polymerase. *In vitro* and *in vivo* transcription, transactivation assays and cross linking experiments have resulted in identification of the specific interaction between the activator protein and the individual subunits of RNA polymerase. These developments have led to the classification of the activator proteins into different groups. Class I activators interact with specific sites in the carboxy terminal of the alpha subunit of RNA polymerase. Examples are the CRP in the case of the *lac* operon, and OxyR, Ada and OmpR. Class II activators, on the other hand, contact the sigma subunit of the polymerase. For instance, the lambda CI repressor protein binds towards the carboxy terminal of the sigma subunit. Another example is the Pho B protein, involved in the regulation of genes concerned with phosphate regulation. A number of transactivators contact either the alpha subunit or the sigma subunit in different systems to activate transcription. For example, at the lacP1 promoter, CRP binds to the alpha subunit whereas at the galP1 and melR promoters, it interacts with the sigma subunit. Besides these two major classes of activators (i.e. activators contacting either alpha or sigma subunits), other classes activators have been identified. The N4SSB, single strand DNA binding protein from bacteriophage N4 activates transcription from late promoters of the phage without binding to DNA. It is found to interact with the carboxyl terminus of beta subunit of RNA polymerase. DnaA protein of *E. coli* is also a positive regulator activation of lambda promoter. Unlike the other activators discussed above, it activates by contacting beta subunit of RNA polymerase.

**Figure 1.** Promoter – polymerase interaction during transcription initiation. RNA polymerase searches for the promoter and binds initially to the -35 element (Kb). In the next step DNA is melted at the -10 region to form a irreversible complex. Upon nucleotide addition ternary complex comprising of DNA, polymerase and RNA is formed prior to promoter clearance.
In addition to the DNA binding domain, many transactivator proteins also have a distinct transactivation domain. Although relatively less is known about the structures of these domains, the mutational analyses of several activators have provided insights into the structure-function of such regions. These regions may function during transcription through protein-protein contacts either with RNAP or with other co-activators. The regions involved in transcription activation may be hydrophobic patches or charged residues. In some cases, the regions involved in transcription activation overlap the DNA binding domain. Thus, as mentioned in the beginning, regulation of transcription activation is achieved by a variety of ways. I will describe below a regulatory system studied in my laboratory for the past several years. These studies have led us to discover new mechanism of transcription activation.

The bacteriophage Mu possesses a unique DNA modification not found elsewhere. The mom gene product of the phage is responsible for the conversion of adenines to N-acetamido adenine by yet an unknown reaction pathway. Due to this modification, the phage DNA becomes resistant to cleavage by a variety of host restriction enzymes and hence mom function represents a novel anti-restriction system. A complex pattern of regulation of this anti-restriction function has been observed (Fig.2). Two host proteins, OxyR and Dam methylase, exhibit contrasting roles in mom gene regulation. A role for DNA methylation in mom gene expression was first described about two decades ago! Dam methylase binds to GATC sequences and methylates adenosine at N$^6$ position. OxyR is the repressor of mom operon and methylation of the GATC residues prevents OxyR binding. Methylation of the GATC sequences is required for mom expression and OxyR essentially serves as methylation blocking factor. Phage Mu gene products, C and Com, function as transcription and translational activators respectively. Four late promoters of phage Mu are transactivated by C protein. Site specific DNA binding property of C protein has been established by electrophoretic mobility shift assay and DNase I foot printing experiments. The protein binds to the specific sequence, located in the mom gene regulatory region, with a very high affinity.

Figure 2. Regulatory region of mom (See text for details)

- ★★★ : GATC sites, Positive regulation
- OxyR : Repressor
- C protein : Transcription activator
- P1-P2 : Overlapping promoters
- -A-A- : T – stretch (DNA distortion)
In addition to the above mentioned findings, a large number of experiments have been carried out to understand C protein structure-function and its role in mom gene transcriptional activation. The protein is a dimer in solution as determined by polarization, gel electrophoresis, gel filtration, cross linking, etc. A variety of experiments indicate that it also binds to DNA as a dimer. Overproduction and large scale purification of the active C protein facilitated studies on protein: DNA interactions. The extremely high specificity of the C protein to its target site on DNA is reflected by a very high binding constant and isolation of protease resistant C-protein:DNA complex. Further, the role of metal ions on the complex formation has been studied. Mg\(^{++}\) ions are important for the protein structure and also for binding to DNA. Mg\(^{++}\) ion mediated conformational changes in the protein are necessary for its binding to DNA. High resolution foot printing studies such as in vivo and in vitro DMS foot printing, Cu-OP probing, methylation interference, and hydroxyl radical foot printing demonstrate that the protein distorts the DNA. The mode of C protein binding to its site is asymmetric with localized unwinding at 3’ end of the site.

Thus, C protein has certain remarkable properties: 1) high affinity binding comparable to that of the lambda cI repressor; 2) Mg\(^{2+}\) triggered conformational change as a prerequisite for binding; 3) axial distortion of DNA, and 4) asymmetric unwinding at 3’ end of the recognition sequence. In addition to the DNA binding motif and a dimerization interface, the protein possibly contains an interacting surface for RNA polymerase for transactivation function. The 5’ and 3’ end deletant as well as point mutant proteins were then generated to determine the DNA binding domain and the oligomerization interface. We have confirmed the DNA binding domain of the activator protein using secondary structure predictions, molecular modeling and site-directed mutagenesis. Our studies indicate that a helix-turn-helix motif towards the carboxy terminal end of the protein is the DNA binding domain and that it is a part of a three-helix bundle. In addition, residues involved in the transactivation function of C protein were also identified and these residues map within or close to the HTH motif. Thus in this activator, DNA binding and transcription activation functions overlap and the domains are not separated from each other. These ‘positive control’ mutants may be responsible for the interaction of transactivator with RNA polymerase. This protein: protein interaction may have a role at subsequent steps of transcription initiation at the promoter after recruitment of polymerase to the promoter. Thus, the C action at mom promoter is likely to be at more than one step illustrated in Fig. 1. This aspect of transcription activation has been examined in detail.

In the mom promoter region there are two divergent promoters -P1 (or P\(_{mom}\)), responsible for mom transcription, and P2 which directs transcription in the opposite orientation (Fig. 2). The -10 and -35 elements of P\(_{mom}\) are away from promoter consensus sequence and the spacing between them is suboptimal. A stretch of 6 T residues just upstream to the -10 element confers an intrinsic curvature to the promoter preventing RNA polymerase binding. The enzyme instead binds to P2. The P2 promoter thus appears to function as a regulatory element for P\(_{mom}\). The C protein, binds to its palindromic sequence located adjacent to -35 element of P\(_{mom}\) and overlapping the -10 element of promoter P2 of the complementary strand. Binding of C with high affinity to its site results in untwisting of the downstream region leading to realignment of the otherwise ‘out of phase’ promoter elements, to facilitate RNAP binding (Fig. 3). This unusual activation mechanism has been confirmed by experiments involving spacer deletion and synthetic promoter constructs. These findings reveal a new mechanism to recruit RNA polymerase to a very weak promoter with an unfavorable configuration for polymerase binding and then initiate transcription. Enhancement of transcription by the transactivator in a C independent promoter mutant
suggested additional downstream function for the activator. Employing a promoter mutant in which RNA polymerase binds without C and a transactivation mutant of C, overall mechanism of *mom* transcription activation is addressed more recently. These studies revealed that C activates transcription at more than one step during transcription initiation at *P*<sub>mom</sub>, to ensure complete transcription activation (Fig. 3).

The transactivator does not influence formation of open complex or its stability after facilitating the polymerase binding. However, at a later step of promoter polymerase interaction, the protein exerts an important role. It enhances enhancing the promoter clearance of polymerase by increasing the productive ternary complex formation. The positive control mutant of C is compromised at this step, confirming the additional downstream role for C in *mom* transcription activation. This unusual multi-step activation may have evolved to ensure irreversibility of the genetic switch during late lytic cycle of the phage.

The complexity of *mom* regulation in relation to eukaryotic systems is particularly striking and hence would serve as an instructive exercise in unraveling complex underlying mechanisms of regulation of transcription initiation and differential gene expression. A regulatory system discovered recently has striking resemblance to *mom* gene regulatory organization. Like the *mom* locus regulation, the major phase variable outer membrane protein of *E. coli* (antigen 43-Ag43) is regulated by Dam methylation. As in the case of *mom* regulatory region, three GATC sites are present upstream of the promoter region and methylation of all the three sites by Dam methylase ensures transcription of Ag43. The
methylation of the GATC sites results in blockage of Oxy R binding. Oxy R functions as a repressor by essentially serving as methylation blocking factor, a phenomenon first observed at $P_{mom}$. Hemi methylated state induced as a consequence of new round of replication results in OxyR binding, thereby inhibiting further DNA methylation and repressing transcription leading to agn43 off state. The presence within the agn43 regulatory region of a sequence identical to that of C binding site at $P_{mom}$ suggests a role for unknown transactivation protein with analogous function. These parallels suggest that the newly discovered mechanisms have evolved much earlier and also employed by the cells as regulatory strategies.

**SELECTED REFERENCES**


**From the Treasurer’s Desk**

All ordinary and student members of the Society are requested to renew their membership, if they have not already done so. Demand drafts payable at Pune may be drawn in favor of Indian Society of Cell Biology and sent to Dr. Surendra Ghaskadbi, Secretary, ISCB, Division of Animal sciences, Agharkar Research Institute, G.G. Agarkar Road, Pune-411004

In case of change of contact information, please e-mail to Dr. Vidya Patwardhan Treasurer, ISCB at <vidyapatwardhan@gmail.com>
Lentiviral vector for gene transfer/therapy

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Originally the idea of Gene therapy envisaged introduction of genetic material in cell/tissue aiming to replace/repair a missing or defective gene to treat a genetic disorder. Gradually the concept also included purposes such as supplementing a gene such as a tumor suppressor/cytotoxic-activating gene to treat a malignancy or an immunostimulatory gene to prevent an infectious disease. However, in all cases, sustained appropriate tissue-specific gene transfer/expression with host safety is the main target of gene therapy. Thus the basic aim is efficient transfer of potentially important/therapeutic gene(s) to defined cell populations. This strategy is a function of two factors. 1] The target cell type concerned and 2] nature of vector used [viral vector or non-viral vector].

Why Viral vectors?

Viruses are a unique class of infectious agents; obligate parasites, mostly of narrow host range, many with small genome size, fast turnover, and short life cycle and they have evolved efficient strategies for transferring their genetic material from one host cell to the next, i.e., the ability to infect the host cells. Some insert their genome in the host DNA and become permanent residents of the infected cells and all its progeny and in many cases the integrated viral genomes retain the ability to produce new viral particles. The criteria for viral vectors are i] efficient transfer [i.e., efficient therapeutic gene delivery], ii] target appropriate cells, iii] sustained gene product delivery, iv] attain in vivo physiological levels of the desired gene product, v] prevention of immune response to viral vector protein(s). Both DNA Viruses and RNA Viruses can be potential vectors for gene transfer.

In DNA viruses the viral genome may be of varying sizes, as small as 3 kb to as big as 200 kb (+). Since large genome can accommodate a large exogenous genome or insert; this may be advantageous but at the same time many viral proteins has a potential of more chances of immune system to target vector proteins. It is more complex to engineer but it can infect various terminally differentiated as well as quiescent cells, thus a broad host range, a major plus point. So far four of DNA viruses have been harnessed for vector development, Adenoviruses; a group of Parvoviruses, namely adeno-associated viruses (AAV) and Herpes simplex virus.

RNA viruses are usually of 7-20 kb in size because there is limit to the higher size of genome. Cells have DNA repair mechanism but not RNA repair, in absence of an inherent system of proof reading (which did not evolve), a very long RNA molecule copying will introduce deleterious mutations; in fact replication of RNA viral genome is accompanied by very high mutation rates. Therefore, a vector derived from a RNA virus can accommodate only small inserts but also have less chance of host immune reactivity. Its small size makes it easier to engineer and can infect usually dividing cells, but random or non-random integration related problems may occur.

Thus, no viral vector is really ideally suited in nature for artificial gene transfer and one has to engineer depending on purpose and expertise available. Systems are selected depending on the requirement such as whether a permanent modification of the host cell genome is the target or a temporary supplementation/correction is needed.
When a permanent correction is the target Retroviruses have several distinct inherent structural/functional advantages that led to their increasing use in gene therapy. The salient features are small genome size; efficient and stable integration (exclusive ability to transform their single-stranded RNA genome into double-stranded DNA that stably integrates into and permanently modifies the host cell genome, i.e., replicate through a DNA intermediate); can only transduce dividing cells; semi-random/random integration. Three strategies provide for gene transcription in these vectors i) linkage of the insert to long terminal repeat (LTR) promoter, ii) use of alternative splicing to express one or more genes from LTR or iii] linkage of gene(s) to internal promoter(s); Self-Inactivating Vector (SIN vector) where the enhancers or enhancers and promoter in the 3' LTR are deleted and construction of packaging cell lines comparatively easy in this vector system. Also they have the unique ability to incorporate envelope proteins from a wide range of heterologous viruses, providing a means for specific cell targeting or foreign antigen display. Among the retroviruses, Lentiviruses are favoured these days for vector development; these are the slow growing viruses (lenti in Latin means 'slow') characterized by a long incubation period to cause the clinical out come and has the ability to infect even non-dividing target cells. They efficiently integrate in genome of non-proliferating cells primarily by virtue of having evolved a complex regulatory and accessory genes system. Most members are highly pathogenic: HIV-1 and HIV-2 (for human beings).

Making a Lentivirus vector

Retroviruses per se encode three major genes, namely gag, pol, and env, which encodes respectively the viral core proteins, the viral replication enzymes including protease, reverse transcriptase, integrase and the envelop protein. Lentiviruses additionally have some regulatory and accessory genes, of which two are important in their replication profile determinations-tat and rev.

A Retroviral or a Lentiviral vector is made into a replication defective construct whereby the engineered construct retains capability to infect and integrate into a target cell but are depleted of protein coding genes resulting in loss multiplication potential and therefore ability to spread to other cells. This short construct, which is devoid of major structural protein coding genes, can be made to accommodate the gene of interest (transgene) under a suitable promoter and used to ferry the said gene for expression in target cells/tissues. Once the construct is ready, it needs a system that will provide all the proteins to package the said construct to make a complete virus but with a replication defective genome since the engineered viral genome will not have viral protein coding genes in it. However for the packaging purpose the structural proteins will have to be complemented. To achieve this, a packaging cell line, usually human embryonic kidney cell line (HEK293), is used and the defective genome containing plasmid is co-transfected with helper plasmids, which will consist of the viral structural/regulatory genes as separate entities under suitable promoters (usually cytomegalovirus-CMV immediate early promoter). Thus, the packaging cells will make a replication defective but integration intact viral RNA genome that will only get packaged (since the nucleotide stretch that is known as packaging signal is kept only on the engineered vector genome) by the proteins provided by transcription of the helper constructs supplied in trans. This manipulation results in production of otherwise morphologically similar virus like the parental strain but the only difference is that these viruses can infect, integrate in target cells but can not produce any progeny being replication defective/lacks all structural protein coding genes. The following cartoon makes a simple representation of the procedure.
In this picture PBS stands for primer binding site, Elongation factor α [EF1α] is the well characterized promoter for the GFP transgene, RRE/ppT region that codes within the env is needed for the regulatory action of Rev regulatory protein for nuclear transport of viral RNA.

Over the years several further modifications have been made and many versatile Lentiviral vector formats have been such as vector with features for positive selection of infected/transduced cells (by antibiotic selection), incorporation of multiple transgene (by multiple promoters/use of internal ribosome entry sites-IRES), FACS sortable infected/transduced target cells (by cis presence of fluorescent protein coding genes-GFP/RFP in addition to transgene), controlled switch on/off version with a drug regulatable system incorporation etc.

Since the virus genome is bounded on either side by LTR that contains integration and promoter activity defining sequences and since practically the integration phenomenon is random/semi-random, one important concern is to make partial deletion in the downstream LTR promoter activity defining stretch. This will allow single replication in packaging cells and subsequent integration but no promoter activity. This is done to ensure that an integration of the vector upstream to a oncogene will not result in activation of the same due to promoter activity of the LTR. Among the gene therapy trials now underway world-wide using viral vectors, majority (almost similar number) of trials are using either retro/ lentivirus or adeno virus based vectors. Initial trials had problems most often due to lack of fine safety factor incorporation in the vector design. Hopefully in near future success will be harvested with current and future improved, safe and efficient vectors. However, apart from therapy as a prospect for its use, these vectors are important tools for research. Using a lentiviral vector one can make gene knock-in or knock-down (by delivery of an appropriate siRNA coding gene as transgene) and develop transgenic cells lines to address specific question of interest.
Stem Cell Research in Drosophila

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Stem cell science in “modern era” is not only important to understand the fundamentals of cell biology but also crucial for the development of regenerative medicine, understanding the aging process, tumor formation and degenerative diseases. By definition, stem cells have the potential for unrestricted or prolonged self-renewal, as well as the capacity to give rise to at least one highly differentiated successor, and with above ability, they are the building blocks of development and permit the maintenance and renewal of tissues throughout the lifetime of an entity. The capability of stem cells to contribute to these processes depends on their ability to divide and generate both new stem cells (self-renewal) and specialized cell types (differentiation). Totipotent stem cells are ones that are competent of giving rise to every cell type in the organism, such as human embryonic stem cells. In contrast, stem cells that are present in the adult organism have a more limited differentiation potential and are most often used for continuous supply of new cells to replace short-lived but highly differentiated cell types, such as blood, skin and sperm. Thus, adult stem cell populations are essential for both normal tissue homeostasis and repair of tissues after wounding or environmental insult.

Although adult stem cells have been identified in a variety of tissues, little is known about the mechanisms that allow for the remarkable, long term capacity of these cells to self-renew and produce cells that will initiate differentiation. The critical choice between stem cell self-renewal and differentiation must be tightly controlled because if too many daughter cells differentiate, the stem cell population could be depleted. On the other hand, unrestricted stem cell self-renewal could expand the number of proliferative, partially differentiated cells in which secondary mutations could arise, contributing to tumorigenesis. Therefore, understanding how the choice between stem cell self-renewal and the inception of differentiation is made might facilitate the expansion of adult stem cells in culture while maintaining its indispensable characteristics.

In vivo, stem cells need specific regulatory microenvironments for its maintenance termed as “niches”. It is suggested that, stem cell number, division, self-renewal and differentiation are regulated by the integration of intrinsic factors with extrinsic cues provided by the surrounding microenvironment (niche). Indeed, is has been demonstrated that many stem cells lose their ability to self-renew when removed from their normal environment, which suggests that the niche plays a major role in controlling stem cell behavior. Thus, among the top priorities in stem cell research is to characterize the microenvironment (niche) “in detail”, and to reveal the molecular mechanisms involved in cross-talk between stem cells and their niches.

Stem cell research and Drosophila

The fruit fly Drosophila melanogaster has been a leading model for biological research for 100 years. The benefits of Drosophila include its short life span (~2 months) ease of culture and the availability of powerful genetic and molecular biological tools. Interestingly, this same tiny fly has also emerged as an exceptional model system for stem cell research. Drosophila stem cell systems have proven to be extremely powerful tool for studying the mechanisms by which stem cell behavior is controlled by the surrounding microenvironment.
and inherent cell fate determinants. Studies on *Drosophila* have also provided a paradigm for the characterization and analysis of stem cells in other systems. Some major contribution include the identification of support cells that constitute the niche, signals that control stem cell self-renewal and proliferation, and molecules that are important for integrity within the niche.

Although stem cell niches have been demonstrated to exist in several *Drosophila* adult tissues, including Malpighian tubules, germlines and midgut epithelium; the adult germline has emerged as a powerful model in which to study how asymmetric stem cell division can be regulated by the surrounding microenvironment. Two stem cell types, somatic stem cells (SSCs) and germ-line stem cells (GSCs) exist in the *Drosophila* ovary. At the anterior end of each ovariole of an ovary, or germarium, are two or three GSCs whose progeny eventually develop into mature oocytes. One major advantage of *Drosophila* GSCs is that their asymmetric division usually always gives rise to one daughter cell that retains stem cell characteristics and one that commits to differentiation. They divide and produce a population of mitotically active follicle progenitor cells which reside in the middle of gerarium. *Drosophila* adult testes also harbor approximately nine GSCs at their apical tip forming a ring that closely surrounds a cluster of post-mitotic somatic cells. Like female germ line, when a male GSC divides, it also gives rise to one cell that retains stem cell identity and one cell, called a gonialblast, commences differentiation.

Studies of the *Drosophila* germline have revealed several important characteristics of stem cell niches those are important for controlling stem cell behavior include: (a) signals originating from the niche regulate stem cell self-renewal, endurance and maintenance; (2) cell-cell adhesions, adherens junctions and gap junctions between stem cells and surrounding supporting cells anchors stem cells within the niche and close to self-renewal signals; and (3) atypical organization of stem cells and niche cells can polarize the stem cells, providing spatial cues towards which stem cells can orient their mitotic spindles that ensures an asymmetric division.

Genetic and transgenic analyses have identified several conserved signaling pathways that function in the germcell to regulate stem cell maintenance, division and differentiation, including the wingless, notch, Dpp, hedgehog, JAK/STAT, insulin and TGF-β pathways. In a recent report, Dicer-1 (Dcr-1), which is essential for generating mature miRNAs from their corresponding precursors, has been demonstrated to be essential for the maintenance of the stem cells (GSCs & SSCs) in *Drosophila* ovary. Interestingly, it has been observed that during *Drosophila* aging the division of the stem cells decreases dramatically, coincident with reduced egg production.

**Concluding remark**

A general depiction of how the stem cell niche controls stem cell number and maintains the balance between self-renewal and differentiation is beginning to emerge from work on *Drosophila* male and female GSCs. These studies have provided a paradigm for the characterization and analysis of stem cell niches in other systems. Interestingly, the male and female *Drosophila* GSCs also provide a unique opportunity to compare two different stem cell systems, particularly since they can be studied in their normal anatomical context. A significant amount of information is now available about the signals involved in controlling germ line stem cell; several exciting questions remain to be answered regarding the somatic stem cell niche, its physical and molecular components and contribution /influence of other signaling network to the maintenance and proliferation of the somatic stem cell population.
Some interesting papers


Contact details of Secretariat, ISCB

Feedback on this issue of ISCB newsletter is most welcome.

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Visit the ISCB website at : http://www.iscb.org.in
XXXI ALL INDIA CELL BIOLOGY CONFERENCE, VARANASI  
(December 14 to 16, 2007): A REPORT

The annual meeting of the Indian Society of Cell Biology, the “XXXI All India Cell Biology Conference & Symposium on Stem Cells: Application and Prospects” was organized at Banaras Hindu University, Varanasi, from 14th to 16th of December, 2007. Dr R Raman was the Convener of the conference. The conference was inaugurated by Dr P Singh, Vice Chancellor, Banaras Hindu University. Dr S K Apte, President, Indian Society of Cell Biology welcomed the guests on the behalf of the society and delivered Presidential address. The conference included Prof J Das Memorial Lecture by Dr V Nagaraja, Indian Institute of Science, Bangalore, 14 invited talks, 21 proffered oral presentations by students and by some of the senior members, 59 poster presentations, an interactive session on research methodologies and the meeting of Executive Committee and General Body of Indian Society of Cell Biology.

FOURTH PROF J DAS MEMORIAL LECTURE

The Prof J Das Memorial Lecture, a prestigious award lecture, was delivered by the distinguished scientist, Dr V Nagaraja, IISc, Bangalore. The session was chaired by Dr S K Apte & Dr S Ghaskadbi (President & Secretary of ISCB, respectively). In his lucid talk, Dr Nagaraja gave an insight into various cellular DNA transactions.

SESSION I

The first session of the conference had four invited lectures and two oral presentations by students. The session was chaired by Dr Joyoti Basu, IICB. Through the first lecture Dr S Galande, NCCS, discussed about the third dimension of gene regulation by a global chromatin organizer and transcription factor, SATB1. Dr D M Salunke, NII, gave a new dimension to antigen-antibody recognition applying thermodynamic and crystallographic approaches. Dr A Ranganathan, ICGEB, demonstrated generation of a novel functional protein by combining a set of DNA hexamer duplexes with a view to identify newer functional proteins and generating inhibitors for protein targets in pathogenic organisms. Dr Somdatta Sinha, CCMB, gave a lucid account highlighting protein modeling towards understanding their structures and function. Through a proffered presentation Ms Ruchi Jain, a student member, illustrated transient association of calcium binding protein 1 of Entamoeba histolytica with actin mediating critical roles in cellular activities, such as phagocytosis; while Mr S M Pandey, DU, demonstrated nucleosome remodeling by the SW1/SNF chromatin remodeling complex obtained from chicken liver nuclear extract.

SESSION II

The afternoon session of the day had one invited lecture and four proffered talks. The session was chaired by Dr T Mukhopadhyay, Panjab U. Attention deficit hyperactivity disorder (ADHD) is a common behavioural disorder in childhood. Dr M Muenke, NIH, Bethesda, USA, demonstrated that a minimal critical region of ~327 kb within the latrophilin 3 gene involved in susceptibility of the disease and that a variant confers protection against the disorder. Dr Sabita K Murthy, Al Wasl Hospital, Dubai, discussed the use of high resolution array-CGH in genetic diagnosis of multiple congenital anomalies and mental retardation in children. Among the three students’ presentations, Mr R Puri, IITK, discussed the interaction and regulation of tau by Lafora disease proteins, Laforin and Malin; Ms Nilambra Dogra,
Panjab U, demonstrated activation of wild type p53 to a very high level by treatment with a tubulin depolymerizing agent in human lung cancer cells resulting in tumour cell death; while Ms Moushami Mallik, BHU, threw light on various pathways through which a non-coding RNA could possibly ameliorate poly Q-induced neurodegeneration in Drosophila.

**SYMPOSIUM ON STEM CELLS: APPLICATION AND PROSPECTS**

The second day of the conference started with the symposium on Stem Cells, chaired by Dr Rita Mulherkar, ACTREC. This included three invited lectures and two proffered talks by student members. Dr Bibha Choudhary, Manipal Inst Regenerative Med, discussed about loss of type II TGF-β receptor in neural crest stem cells leading to cardiovascular malformation and aneurysm. Dr S Ghaskadbi, ARI, described his laboratory’s approach to study stem cells in ‘primitive’ organisms in his talk on ‘Insulin signaling in pattern formation: A search for receptor tyrosine kinases in hydra’. On the other hand Dr A Rai, BHU, showed how limbal stem cells are transplanted to cure chemical injuries of the cornea. Patients transplanted with stem cells regained normal vision. Through the proffered papers Mr P Chandrashekar, CCMB, showed that argonaute 2 deficient murine embryonic stem cells are capable of self renewal as well as differentiation; while Ms K L Surekha, ARI, demonstrated positive enhancement of angiogenesis in chorioallantoic membrane of chick embryo by insulin and FGF2.

**SESSION IV**

The post tea session of the second day had two invited and two student presentations under the chair of Dr K Majumdar, CCMB. In this session Dr R Tuli, NBRI, gave a lucid overview of nucleosomal organization, histone modifications and regulation of gene activity. Dr Aparna Duttagupta, Hyderabad U, threw light on the regulation of hexamerins, which account for ~80% of haemolymph proteins, by the 20-hydroxyecdysone during various stages of rice moth development. Mr H M Reddy, CCMB, through proffered paper demonstrated extensive alternate splicing of a newly identified non-coding RNA coded by the gene located in Yq heterochromatin and discussed the role of these RNAs in gene regulation. On the other hand Mr P Lakshminarasimhan, NCCS, showed transcriptional and cell cycle regulation by the tumour suppressor protein, SMAR1 upon DNA damage by stabilizing p53 sequestering MDM2.

**SESSION V**

The post lunch session of the day included two invited lectures and four student presentations. Dr V Radha, CCMB, chaired the session. Growing pollution is of great concern. Through a study conducted on Drosophila, Dr D KarChowdhuri, ITRC, showed that some of the industrial waste leachates may affect fertility and fecundity in the exposed organisms. By inactivating an anti-sigma factor in Azospirillum, Dr A K Tripathi, BHU, demonstrated activation of a cryptic carotenoid biosynthetic pathway. Through students’ presentations, Mr T L Pavankumar, CCMB, showed that RecBCD pathway of DNA repair is essential for growth of the Antarctic Pseudomonas species at low temperature; Mr A Bhattacharya, IICB, demonstrated the importance of phosphodiesterases in the differentiation of specific infective stages in Leishmania; Mr P Kumar, DU (South Campus), discussed characterization of five members of ribulose-1, 5-biphosphate carboxylase/oxygenase enzyme of cotton, while Ms Vidisha Tripathi, BHU, discussed the role of Cyp19A1 aromatase in gonadal differentiation in Calotes.
SESSION VII

The morning session of the third day had three invited lectures, one student presentation and a technical lecture. The session was chaired by Dr Shanti Chandrashekaran, IARI. Dr B M Meclher, German Cancer Research Centre, Heidelberg, established a link between arginine methylation and localization of germ line determinant proteins to their subcellular compartments during oogenesis in *Drosophila*. On the other hand Dr P Sinha, IITK, demonstrated the roles of Fat tumour suppressor in cell sheet migration during morphogenesis in *Drosophila*; while Dr A Mukherjee, BHU, lucidly discussed the regulation of Notch signaling by its interacters. Mr P Singh, BHU, a student member, gave evidence for population sub-structuring and minimal gene flow in natural populations of *Drosophila ananassae* in India. Through a technical lecture Zeiss India, showed new versions of confocal microscopes and developments in imaging.

SESSION VIII

The pre-lunch session of the day chaired by Dr M M Chaturvedi, DU, had one invited lecture and four proffered talks. After wounding levels epidermal hyaluronans are greatly increased in the skin and this increase promotes wound healing. Dr E Maytin, Cleveland Clinic Foundation, Cleveland, USA, showed that the accumulation of hyaluronans require epidermal growth factor receptor mediated signaling. Through proffered papers Dr Judith A Mack, Cleveland Clinic Foundation, further demonstrated that elevated Hoxb13 expression influences epidermal differentiation, lowers hyaluronan levels and wound healing process; Dr S Anand, from same lab, discussed about the emergence of photodynamic therapy for cancers of various origins; Dr A Kumar, IISc, threw light on the genetic loci involved in primary microcephaly in the families from southern parts of India; while Dr R Gope, NIMHANS, presented the cases of brain tumour specific alterations at p53 gene locus causing altered expression and phosphorylation of p53 gene product.

SESSION IX

An interactive session on ‘Research Methodologies : Problems and Solutions’ was held under the chairmanship of Dr S C Lakhotia, BHU. In this session Chairperson invited specially the student participants to present innovations in research methodologies made by them or the problems faced at any step in their research work. Ms Richa Arya, BHU, stimulated the audience with the innovative technique that after pealing the dried nail polish from the surface of *Drosophila* eye and examining the peal under the microscope gives comparable detailed image of the ommatidial organization in compound eye as viewed under scanning electron microscope. Students came up with several problems and obtained answers and suggestions from other participants.

POSTER SESSIONS:

SESSIONS III, VI AND IX

A total of 59 posters were presented on the diverse areas of Cell Biology during the poster sessions. All posters were on display on all the three days and sufficient time slots were allotted to the posters. A large number of posters were from student members of the society. Most of the posters, especially the students’ posters, were of high quality. Since these sessions gave an opportunity to all the interested participants to search the posters of their
interest and to discuss the work at length, good interactions between the presenting author and the participants were seen. As large number posters were presented, the highlights of each of them are not being presented here.

AWARDS TO STUDENT MEMBERS

On the whole the deliberations through platform and poster were stimulating and highly educating. From thirteen oral presentations by student members, the paper entitled “Lafora disease proteins laforin and malin interact with and regulate tau, a microtubule associated protein involved in Alzheimer’s disease” by Mr R Puri, Indian Institute of Technology, Kanpur, was selected for Prof A S Mukherjee Memorial Award; while the paper entitled “RecBCD pathway of DNA repair is essential for growth of the Antarctic Pseudomonas syringae Lz4W at low temperature” by Mr. T L Pavan Kumar, Centre for Cellular and Molecular Biology, Hyderabad, received Prof V C Shah Award and the paper entitled “The non-coding hsrc transcripts modulate polyglutamine pathogenesis in Drosophila through multiple cellular targets” by Ms Moushami Mallik, Banaras Hindu University, Varanasi, received the Conference Award.

Out of 44 posters presented by student members, the poster entitled “Hsp60D, a member of the Hsp60 family, is a novel modifier of cell death in Drosophila” by Ms Richa Arya, Banaras Hindu University, Varanasi, was adjudged Prof V C Shah Award; the poster entitled “Enrichment of islet like cells from human bone marrow for cell replacement therapy in diabetes” by Ms Smruti M Phadnis, Agharkar Research Institute, Pune, was given Prof B R Sheshachar Memorial Award; the poster entitled “Effect of Gamma radiation on Indian tropical midge Chironomus ramosus: DNA and protein profiles” by Mr K Datkhile, University of Pune, Pune, received Dr Mansi Ram Memorial Award, while the paper entitled “Hybrids of sibling species of Drosophila are developmentally as stable as their parents” by Ms C Vishalakshi, Banaras Hindu University, Varanasi, received the Conference Award.

Report prepared by

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Nominations for the

Twelfth Prof. S.P. Ray-Chaudhuri 75th Birthday Endowment Lecture

Dear Member,

The Indian Society of Cell Biology has instituted the "Prof. S.P. Ray-Chaudhuri 75th Birthday Endowment Lecture" as a mark of its respect to Professor S.P. Ray-Chaudhuri in recognition of his immense contributions to Cytogenetics and Cell Biology. Nominations are invited for the Prof. S.P. Ray-Chaudhuri 75th Birthday Endowment Lecture to be delivered at XXXII All India Cell Biology Conference to be held at Agharkar Research Institute, Pune in December 2008.

Eligibility for Nomination: The person to be nominated need not be a member of the Society when nominated but may be requested to become a member in due course of time. The person to be nominated will ordinarily be an Indian citizen at the time of nomination/selection and should be an eminent scientist who would have made outstanding original contributions to Cell Biology or contributed substantially to growth of the subject in India.

Please send the nominations in the enclosed proforma so as to reach us by 31st May 2008. The bio-data of the nominee may be sent by email to indiansocietyofcellbiology@gmail.com with a copy to ghaskadbi@gmail.com.

With best regards,

Yours sincerely

Surendra Ghaskadbi
Secretary, ISCB
Name & Address of the member making the nomination:

Nomination

I wish to nominate

....................................................................................................................................................
(address........................................................................................................................................
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for the twelfth PROF. S P RAY-CHAUDHURI 75TH BIRTHDAY ENDOVENTMENT LECTURE I have obtained consent of the nominee for the purpose. The bio-data etc of the nominee are enclosed herewith.

Please send a soft copy of the nominee’s bio-data by email to indiansocietyofcellbiology@gmail.com with a copy to ghaskadbi@gmail.com.

Date                                                       Signature of the nominating member
Crossword Puzzle

Solve this Biology crossword puzzle and submit your entries by post to the secretariat in Pune by 31st August 2008.

A maximum of 3 all-correct entries will be chosen by draw of lots. Prizes (Rs. 200/- each) will be awarded at the XXXII All India Cell Biology Conference at Pune in December 2008.

This contest is open only to ISCB members.
Clues:

Down:
1) Discovered mitosis (8)
2) First type of tissue to evolve (10)
3) Shared a Nobel prize with Cajal (5)
4) Glaciate and rupture (6,8)
9) Type of light microscopy that accentuates edges (Abbr.) (3)
11) ‘Short tail’; developmental gene (9)
12) Vegetable name; genetic element (3)
14) Eukaryotic flagellum (12)
15) The main axis of a compound leaf (6)
17) Morphologically related but evolutionarily unrelated organisms belong to a ________ (5)
18) ‘Devour oneself’ (9)

Across:
5) Useful and functional we appear from afar; but relics are what we really are! ..... Part of genome (11)
6) Together we are regulated to work the best; if one goes down, so do the rest ..... Part of genome (6)
7) Induces cell division (7)
8) ‘Time-giver’ (9)
10) Earmarks for destruction... What molecule? (9)
13) At the 3’ end of an mRNA (4)
15) The most abundant enzyme on earth (Abbr.) (7)
16) Sega’s videogame character; morphogen (Abbr.) (3)
18) Made from red sea-weed; used in microbiology (4)
19) I’m a type of DNA and I can directly code; because of me, the central dogma the scientists rewrote (4)
21) One is added, one is lost; and to polymer size there is no cost! ..... What process if this? (12)
25) ‘Japanese biker’; patterning gene (7)
26) ‘Falling from’... greek (9)
27) ‘Bedraggled’; protein in Wnt signaling pathway (Abbr.) (3)
28) A man I maketh not! _________ hormone (4,9)

The ISCB is grateful to Tanvi Sinha (sinha.tanvi@gmail.com) and Shahnaz Masani (smasani@gmail.com), students of M.Sc. Zoology, University of Pune.
Abstract

Adult murine cardiomyocytes provide an excellent model system for the study of cardiovascular diseases, because the heart’s striated muscle cells represent the functional contractile unit of the circulatory system. Methods to isolate cardiomyocytes have traditionally relied upon mechanical mincing and enzymatic treatment of ventricular tissues, followed by the use of cell strainers, but this risks introducing contamination to the sample. These methods often yield mixed populations of cells making analyses of discrete populations difficult. Here we report an improved method for the isolation and purification of cardiomyocytes from endogenous cardiac stem cells (CSCs). This technology provides a reliable and reproducible method for the isolation and culture of adult CSCs, and should be suitable for a wide array of biological applications.

Introduction

The isolation and expansion of cardiac stem cells opens new opportunities to the field of cardiac regenerative medicine. CSCs have recently been isolated from human and murine tissues based upon cell biomarkers (Sca-1, TeRT, c-Kit, side population), demonstrating the presence of a non-circulating stem cell niche within the myocardium that is estimated to account for 11-14% of the total cell population. These cells appear to be bi-potent in their capacity to form cardiomyocytes and vascular endothelial cells. Moreover, preliminary engraftment studies suggest that these cells are ideal candidates for future research on cardiac regeneration. However, the rarity of CSCs coupled with the complex isolation procedure is a significant challenge for the advancement of the field. To overcome this obstacle, we have developed an easy-to-use cell isolation kit capable of obtaining a high yield, pure population of CSCs. This advancement allows investigators to obtain a significantly greater number of CSCs for their studies without the need for time consuming, complex protocols and expensive cell sorting equipment.

Results

Isolation and purification of murine CSCs using the millipore CSCs Isolation Kit (Cat. No. SCR061) begins with surgical resection of the heart from the thoracic cavity. Heart tissue is minced and pooled from five separate mice per collection and a cell suspension is created through gentle agitation in our dissociation buffer. A novel application of our isolation kit is the use of the self-contained Steriflip® filtration device, which allows for the separation of larger volumes of cellular material with the added advantage of maintaining a sterile environment ensuring sample sterility, an option that open top gravity flow filter devices can not guarantee.

The study used a 100 µm nylon mesh vacuum driven Steriflip device that allows the passage of cells while retaining large tissue clumps. This improves recovery and lessens time to collect the isolated cells.

Figure 1. Discrete cell populations can be isolated from ventricular heart tissue through differential gradient centrifugation. (A) Representative photos depicting heterogeneous cell populations present in the lower phase before centrifugation and a pure CSC population present in the upper phase after centrifugation. (B) Purity of differential gradient isolated CSCs as determined by flow cytometry analysis for the stem cell marker Sca-1.
A major advantage that our system provides is the rapid purification of Sca-1 positive CSCs without the need for expensive cell sorting equipment. This is accomplished through the use of differential gradient centrifugation (Figure 1A). To validate the purification method, we harvested five C57/Bl6 mouse hearts per triplicate isolation and performed the collections as described above. Purified samples were subsequently labeled with Sca-1-FITC conjugated antibodies and cells were analyzed using a FACSCalibur flow cytometer. Isolations yielded an averaged $1.2 \times 10^6$ cells/ml with a Sca-1 purity of $69\%$ (Figure 1B). This method appears far superior to the traditional method of dissociation and filtration that yield a heterogeneous population composed of only $11-14\%$ Sca-1 positive cells.

Purified CSCs can maintain a mesenchymal stem cell like phenotype (Figure 2A) during in vitro culture. moreover, when regular growth media is replaced with differentiation media, CSCs undergo a phenotypic change indicative of mature cardiomyocytes (Figure 2A). labeling cells for filamentous actin (F-Actin) highlights the transition from stem cell to cardiomyocyte, with long striated myofibrils present in the differentiated population (Figure 2B). Both isolation of adult cardiomyocytes and differentiation of embryonic stem cells into cardiomyocytes have been problematic due to the limited number of cells one can obtain. The millipore CSC isolation kit provides the advantage of not only purifying greater number of cells, but also allowing expansion of the original population as needed. Isolated cells can be expanded in culture using defined growth media for periods of up to two weeks before quiescence (Figure 2C), providing the opportunity for additional experimental analysis.

Isolated cells were cultured for 1 week and characterized. Immunocytochemical staining demonstrated a ubiquitous Sca-1 labeling, as previously shown through FACS analysis. moreover, these cells concurrently stained for the stem cell marker, telomerase, suggesting the retention of their stem cell characteristics (Figure 3A). Furthermore, CSCs maintained in culture continued to proliferate and stained positive for Ki67 (Figure 3A).

To establish the differentiative capacity of isolated CSCs, we plated cells on poly-L-ornithine coated slides and maintained them in differentiation media for a period of 12 days. Samples were then fixed and stained with markers for mature cardiomyocytes. Cells showed strong immunoreactivity to cardiomyocyte markers (Cat. No. SCR059), Troponin I, Desmin, and Actinin (Figure 3B). Collectively, these results demonstrate an efficient isolation of CSCs that are capable of expansion differentiation when treated with the appropriate media in vitro.
efficient high throughput isolation of cardiac stem cells has remained elusive. This obstacle has slowed progress on the development of therapeutic applications of endogenous CSCs. We show here how millipore's CSC Isolation Kit enables researchers to obtain significantly greater numbers of cells for use in their experimental applications. Our novel isolation and purification approach, coupled with defined growth media, permits pure CSCs to be isolated from ventricular tissue and expanded as needed. Using our defined differentiation media, cardiomyocytes can be efficiently generated. The dynamic capabilities of this kit enable advancement of cell and molecular biology related to both cardiac stem cell and cardiomyocytes.

references

millipore products

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<tr>
<td>Cardiac Stem Cell Isolation Kit, 5 isolations</td>
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A. Undifferentiated

b. Differentiated Cardiomyocytes

![Image](image)

Figure 3. Cultured CSC retain their stem cell characteristics and efficiently differentiate into cardiomyocytes. (A) one-week cultures of purified CSCs ubiquitously express stem cell markers Sca-1 and telomerase, while remaining in a proliferative state as determined by KI67 immunoreactivity. (B) Differentiated CSCs express mature markers for cardiomyocytes (Troponin I, Desmin, and Actinin).
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