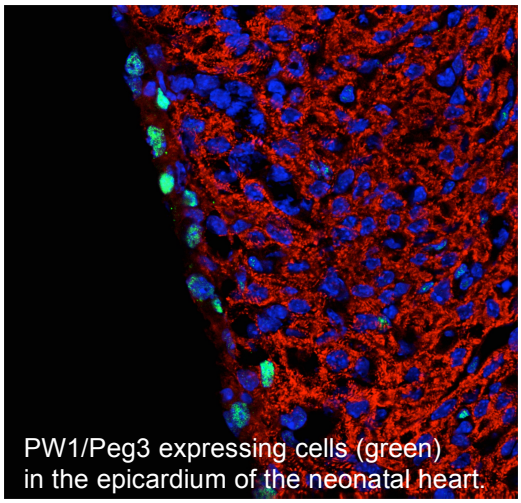
Identifying the molecular and cellular processes contributing to cardiac repair and regeneration… …Leveraging them to restore tissue function



PW1/Peg3 expressing cells (green) in the epicardium of the neonatal heart.

What is Cardiovascular disease (CVD)?

Blockage of a blood vessel, due to age, diet, lifestyle behaviours and underlying genetic conditions results in oxygen being restricted to the surrounding tissue; which then dies.

In the adult heart it is the cardiac muscle tissue that dies, which is a terminally differentiated tissue. In other words, when the muscle dies there is virtually nothing to replace it, a scar forms, and the heart works less well.

This perpetuates stress on the heart inducing more tissue damage.

The disease is a slow, progressive vicious cycle and permanent.

At a minimum it drastically reduces the quality of life, at a maximum it ends life.

Multiple different stem cells have been described in the adult heart; We are defining the relationship between them and translating the insights into therapies

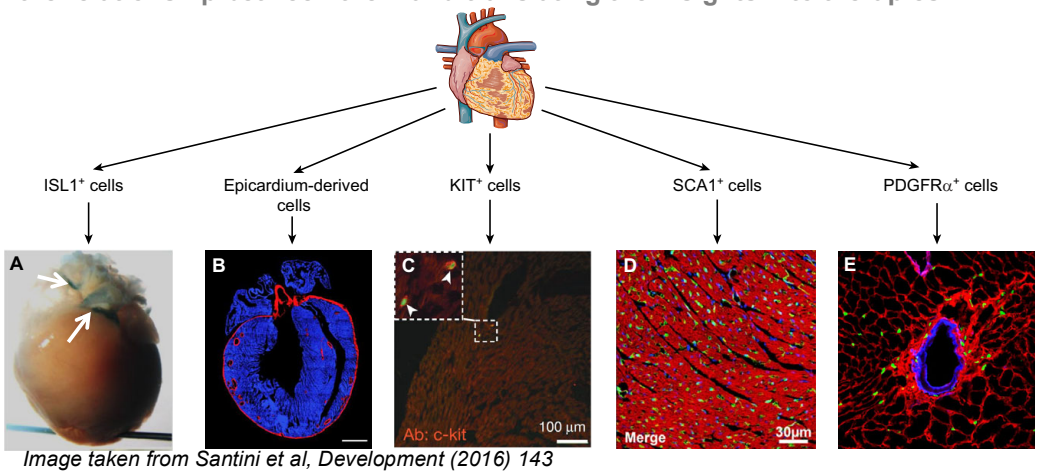


Image taken from Santini et al, Development (2016) 143

CardioStemNet is a Transatlantic Network of Excellence, funded by the Fondation Leducq. It is coordinated by Toren Finkel (NIH, USA), North American Coordinator and David Sassoon (UPMC, France), European Coordinator, partnering with Richard Harvey (Australia), Thomas Braun (Germany), Jean Sebastien Hulot (France), Nadia Rosenthal (United Kingdom) and Roger Hajjar (NY), Jason Kovacic (NY) and Mark Sussman (San Diego) (all United States of America).

How can a global academic network help solve CVD?

CardioStemNet, now two and half years old, represents 9 globally recognised teams from 5 countries on 3 continents, with an R&D headcount of nearly 20 active researchers. Each team has made seminal contributions in their field which when integrated into one-network represents a unique critical mass of laboratory and clinical infrastructure and equipment, highly trained personnel, expertise and insight. This is being focused on addressing a key question in cardiac repair, performed via 25 different portfolio projects, some of which are illustrated here as project case studies.

Cumulatively, establishing such a network, if it was from scratch, accounting for infrastructure needs, expertise and experience would require start-up costs alone of over $30 million. By generating a network and with the R&D financial support of the Fondation leducq, these start-up costs are circumvented and the network therefore free to commit to the R&D endeavour very quickly.

Critically, there is an urgent need for new pivotal knowledge in the cardiac field; irrespective if the knowledge is truly fundamental in nature, offering insights on mechanisms or signaling pathways; more applicable identifying new targets or clinical, suggesting improvements to existing cardiac repair strategies, it will all have impact.

Funded by the Fondation Leducq

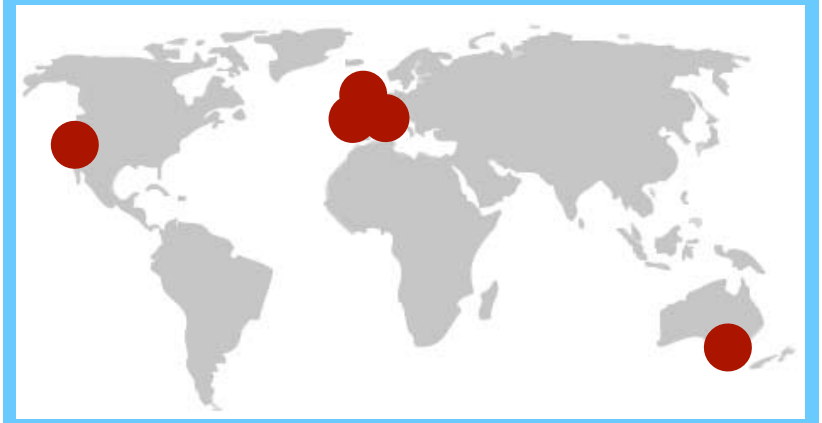
Deciphering the molecular components

Due to the complexity of the cellular composition in the adult mammal, it is unlikely that a suitable solution to treating CVD's will be linked to a single cell type or new drug and will need to integrate or mimic key internal signaling instructions; it is for this reason that we are exploring molecular signaling, originating from developmental signals, growth factors, environmental signals or epigenetics which could be exploited to re-programme the diseased tissue and restore function.

Teams in the USA, working with teams in Germany, the UK are looking at exploiting the capacity of neonate cardiac tissue to repair and regenerate spontaneously leveraging insights on stimulating developmental signals.

Complementing this, the team in Australia, in a skills development collaboration with the USA teams are looking at the signaling pathway induced by hypoxia. Growing mammalian fetal tissue, which has a high regenerative capacity, is in an almost permanent hypoxic state. Hypoxia stabilises the regulatory factor Hif1a, which activates gene programs for blood vessel formation, and a glycolytic metabolic state that protects stem cells against oxidative damage and capacitates them for cell division.

Finally, significant effort across 5 of the teams on all 3 continents is assessing the role of the Pw1 gene. This is a gene that is found expressed in all adult stem cells examined to date. As such, its expression in the heart is of keen interest given that the existence and functionality of cardiac stem cells remains a topic of intense debate.



Project case study: Role of Pw1 in the heart

Lead by the Sassoon group in France, working together with teams in France, the United Kingdom, Australia and the USA, using the Pw1-reporter mouse, we have determined that the predominant population of Pw1-expressing cells in the adult heart display a similar profile to a stem cell population identified by their PDGFR/c-Kit expression, in which these cells are a subpopulation.

We have found that in contrast to other tissues where they represent a reservoir of blood vessel progenitors that are specifically activated to form vessels in response to injury, in the heart they form the cellular components of the cardiac scar following a heart attack. We are presently testing whether the fibrogenic response can be attenuated in vivo by dissecting the molecular and cellular responses to cell stress in these cells. We find that mice which carry a mutation in the Pw1 gene do not undergo pronounce fibrosis in response to myocardial infarction providing proof-of-principle that we can develop a pharmacological approach to mimic the Pw1 mutant scenario.

CVD – The numbers

Updated 2016 figures from the WHO have indicated that over 17 million people per year die from a cardiovascular disease (CVD), representing nearly one third of all deaths worldwide. Greater than 75% of those deaths occur in low and middle-income countries.

More people die from a cardiovascular disease than any other cause, and in most cases the disease could be prevented by addressing lifestyle behaviours. In the United states alone over 2000 people die a day from CVD; that's one person every 40 seconds.

The societal and personal costs for caring for and supporting patients with cardiovascular diseases are enormous: at a last analysis the direct and indirect costs of CVD totaled more than 320 billion dollars in the United States.

The exception to behavioral change is the increased prevalence of degenerative diseases with the growing aged population, which has stimulated a growth in the need for effective and widely applicable therapies.

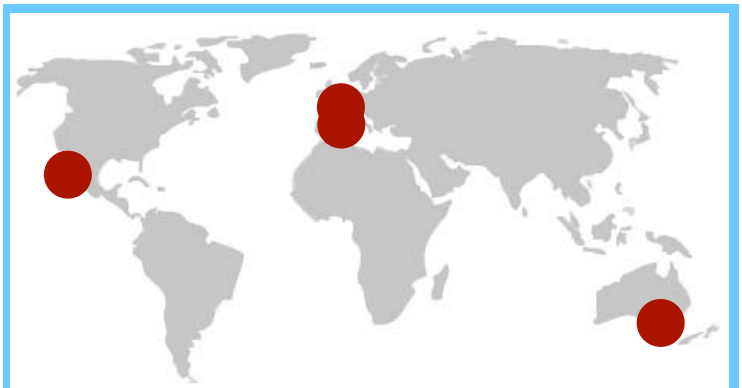
Today there are 600 million people in the world aged 60 years or over, and this will double by 2025 and reach 2 billion by 2050. Degenerative diseases, in many cases not directly related to CVD present comorbidities that result in CVD occurring.

Untangling the cellular component

As illustrated above, the cellular dissection of the different compartments of the growing, degenerating and regenerating cardiac muscle is extremely diverse. The cellular diversity is in fact far more diverse and very dynamic; including the complete plethora of cells that exist in the blood vessel structure and the immune system, which are the first responders to tissue damage. Each cell type has a crucial role to play; the understated characteristics of these responses, which at first glance are natural, in the context of a vicious cycle of tissue degeneration can in fact lead to the exacerbation of the disease.

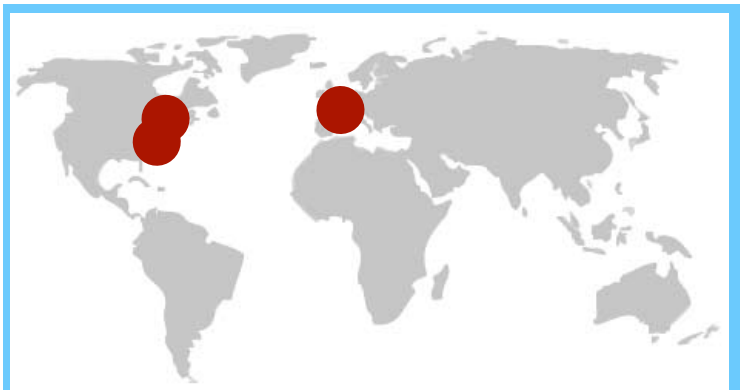
Teams in the USA in collaboration with teams from France are currently exploring stem cell paracrine signaling (cell to cell) to nearby adult cells including their roles in regeneration of contractile or "beating" heart cells and replacement of damaged myocardial tissue. The significance of this project is driven by the clinical relevance of identifying underlying mechanisms of emerging cell therapies for myocardial injury.

Essentially, traversing the cardiac to vascular structures represents a key to generating long term benefit; this is being addressed via research on endothelial metabolism and transition.



Project case study: Developing PDGF

Lead by the Harvey team, collaborating with teams in France, London and the USA, we have focused on identifying factors expressed in the heart that keep stem cells in pristine condition, and factors that modify their behaviour upon injury, may allow us to control stem cell action therapeutically. We have defined one such factor – platelet-derived growth factor (PDGF) – that can help bring stem cells out of their dormancy into a higher metabolic state, capacitating them for growth and differentiation. PDGF therapy has been trialed in mice with myocardial infarction, with beneficial effects on heart repair, and is now being trialed in pigs. To be responsive to PGDF the cells need to express the receptor for the factor, and dissection of this activity may reveal mechanisms of action and the precise target population being affected. In combination with another known stem cell marker, c-Kit, we are working on better understanding the precise stem cell target to elucidate the precise characteristics of action and response.



Project case study: Endothelial metabolism and endothelial cell fate

Lead by the Finkel team at the NIH, working together with teams in New York and France we have been exploring whether substrate utilization can alter cellular fate. Most cells can generate ATP from a variety of substrates such as glucose, amino acids and fatty acids. These substrates are broken down in acetyl- CoA, which can then enter the TCA cycle in the mitochondria to generate ATP. Relatively little is known about endothelial metabolism and how it regulates endothelial biology. We have recently found that inhibiting endothelial fatty acid oxidation (FAO) triggers a profound morphological change in endothelial cells and makes them resemble fibroblasts. This process is called endothelial-to-mesenchymal transition (EndoMT). We have been able to show that if we genetically disrupt FAO we can trigger EndoMT. EndoMT has been linked increasingly to a number of pathological conditions including chronic kidney disease, pulmonary hypertension and atherosclerosis. Our data suggest new strategies that might combat this condition revolving on manipulating endothelial metabolism.

Integrating the molecular and cellular

Schematic of EndoMT role in disease progression

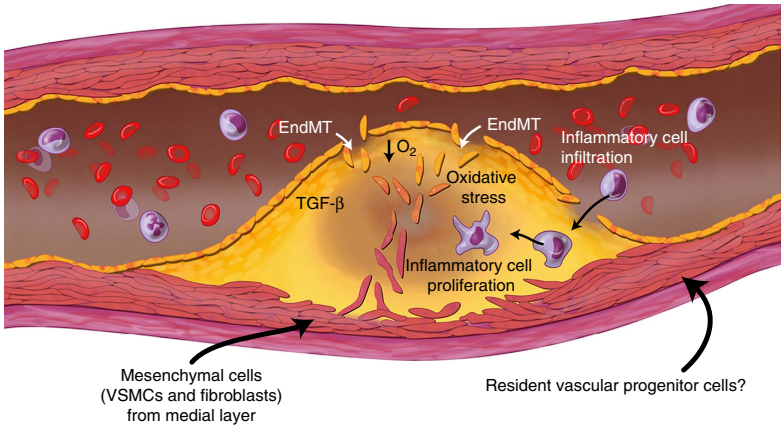
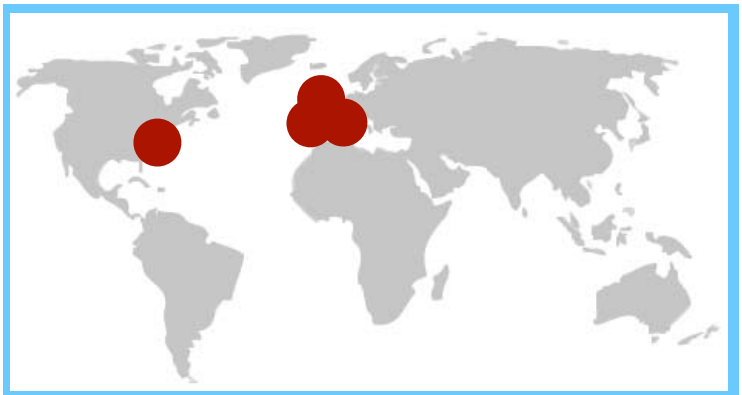


Image taken from Evrard et al, Nat. Comms (2016) 11853



Project case study: Molecular mechanisms of cell differentiation

Lead by the Braun Group at the Max Planck in Bad Nauheim, in collaboration with teams from the NIH USA, France and the United Kingdom, this project is focusing on the mechanisms that alter the differentiated state of cardiomyocytes (cardiac muscle cells), processes that regulates proliferation of cardiomyocytes and on secondary consequences of cardiomyocyte dedifferentiation. We found that the differentiated state of cardiomyocytes requires repression of a specific growth factor receptor by small nucleic acids. Inactivation of the nucleic acids in adult cardiomyocytes dramatically enhanced the growth factor signaling and resulted in cell cycle entry of a substantial portion of adult cardiomyocytes. We also observed that local oxygen concentrations within the developing heart serve an important function to regulate the balance between cardiomyocyte proliferation and differentiation; Hypoxic responses repress expression of the critical genes. We assume that this repression following episodes of hypoxia account for a large degree of congenital heart defects.

Prior to making the pivotal decision of moving insights and innovations into preclinical and clinical development, it is necessary to reintegrate the knowledge obtained, back into existing models and concepts and compare experimental benefits to known standards and indicators of repair, following injury.

Teams from Australia, Germany and the USA have assembled mouse reagents to genetically tag the majority of immature, potentially stem cells in adult heart and are mapping their descendants in health and disease, and in models of heart regeneration.

The projects lead by Finkel and Sassoon are highly complementary, approaching the same question but from opposite sides and the teams and efforts are starting to dovetail around endothelial cell fate, renewal and turnover.

Heart tissue renewal and turnover might be driven by cardiac stem cells, proliferation of immature cardiac muscle after partial loss of cellular functions (dedifferentiation) or a combination of both. We are looking at the mechanisms that change the state of cardiac muscle, signaling that regulates their proliferation and on further consequences of their activity.

Extending this, analysing the canonical stem cell markers to understand CSC relationships, and establish lineage distinctions, is being performed in San Diego, which will provide insight into population heterogeneity. We are also looking at the chromosome content of cardiac stem cells, as this is a theme of regenerative biological life is cellular mononuclear genome duplication or polyploidisation.

Interlocking networks

The Fondation Leducq supports numerous ongoing networks (see here)

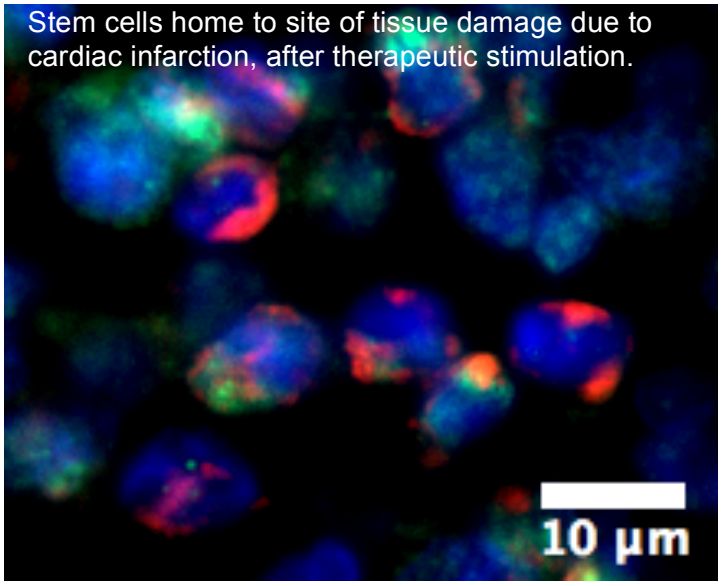
As well as internal network collaborations, there are also inter-network collaborations, meaning we do not work in isolation.

For example, from the Icahn School of Medicine at Mount Sinai, Jason Kovavic is part of this network, while his colleagues Eric Schadt and Johan Bjorkegren work in a different network focusing on Coronary Artery Disease.

Data and knowledge have been exchanged giving rise to new insights, publications and avenues to explore; this is critical for cutting edge innovation and advances.

"A fundamental driving force in the cardiovascular therapeutic field is the realization that pharmaceuticals can only go so far in the treatment of cardiovascular diseases. With increasing complexity of the genetic makeup and the role of multiple factors in the etiology of cardiovascular diseases, there has been a drive to think beyond the ordinary and consider the use of technologies such as tissue engineering and stem cells in managing cardiovascular diseases and leading to treatment of the disease."

Frost and Sullivan
'Advances in cardiovascular therapy'



Stem cells home to site of tissue damage due to cardiac infarction, after therapeutic stimulation.

New therapeutics

The development of novel therapeutics is a very complex, lengthy and expensive process, which requires a multitude of expertise, the majority of which is not linked to laboratory research and knowledge generation.

The previous sections have illustrated examples of some of the initiatives we have taken to identify new innovations, which can lead to new opportunities for therapeutic intervention.

Significant effort is focused on the application of innovations related to growth factor manipulation. Most innovations leading to effective medicines are based on incremental insights; we have illustrated such examples in the project case studies through strategies looking at the application of growth factors.

Beyond this, we have also taken new avenues based on the insights we have obtained from the fundamental molecular and cellular research. Early stage research performed by the two teams in France have identified new populations of stem cells that represent up to 1 in 8 cell in the infarcted heart. These cells seem to act as signaling hubs therefore creating a microenvironment to influence the surrounding cells in the repair process.

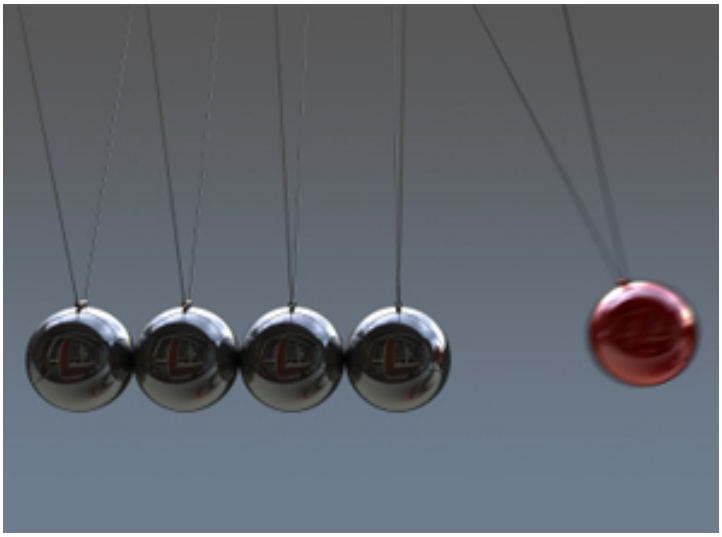
We are currently characterising the signals sent by these cells to identify new factors that can be turned into therapies for the diseased heart, and looking forward to applying those insights.

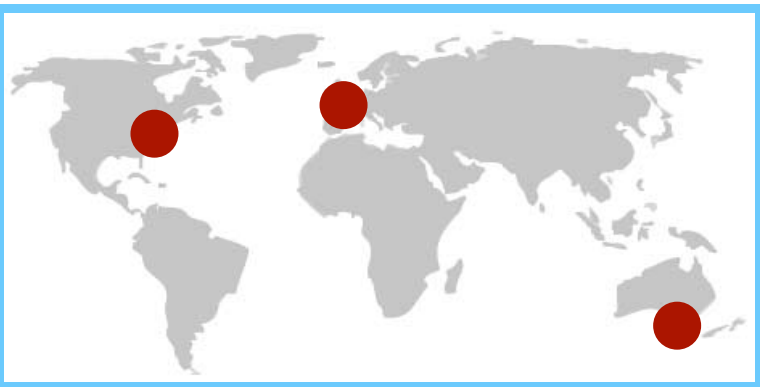
What's next?

CardioStemNet has two and half years more to run. In the first half of its existence it has already given rise to significant progress in generating new knowledge for untangling the biological processes that occur during disease progression from the initiation of disease to those that happen during the full blown pathology.

These have been reported at international meetings and in esteemed journals such as Nature Medicine, Nature Communications, and Development; and we anticipate making more seminal contributions in the coming years.

We will continue to move our insights and potential new innovative therapies towards and into the clinic for human benefit, which is becoming increasingly dependent on academic expertise, insight and infrastructure





Project case study: Growth Factor Therapeutics

Lead by the Hajjar Laboratory at the Icahn School of Medicine at Mount Sinai (NY, USA), in collaboration with local teams, and teams from Paris and Australia, this project is investigating the role of endogenous cardiac stem cells found within the adult heart in cardiac repair after myocardial infarction. We have revealed that by adenoviral gene delivery of Stem Cell Factor, we can stimulate these stem cells resulting in their proliferation and ultimately improved outcomes in a translational model of infarction. We are developing a novel delivery platform that could result in faster stimulation of these stem cells, thus potentially preventing sub-acute damage that occurs after an infarction. On microscopic examination of the heart, stem cells identified by several markers are shown to home into the site of damage after infarct. These marker patterns indicate that a diverse population of stem cells can be induced with a single gene to improve clinical outcomes.

'A comprehensive understanding of the cellular, molecular and metabolic controls that govern and restrict mammalian cardiac regeneration is needed and essential for the rational development of new therapies for acute or chronic heart disease'.

For more information on the network activities

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